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L1 28 STAIN?(5A)SPERM?
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1. 5,021,244, Jun. 4, 1991, Sex-associated membrane antibodies and their use for increasing the probability that offspring will be of a desired sex; Glenn F. Spaulding, 424/561; 435/2; 530/350, 387 [IMAGE AVAILABLE]

US PAT NO: 5,021,244 [IMAGE AVAILABLE] L1: 1 of 28

ABSTRACT:
A method of sorting living cells based on DNA content. Mammalian sperm subpopulations enriched in X- or Y-sperm. X- and Y-enriched sperm-plasma-membrane vesicles. Substantially pure sex-associated membrane (SAM) proteins. Antibodies binding to X- or Y-SAM proteins, essentially free of antibodies binding to Y- or X-SAM proteins respectively, or to the H-Y antigen. Semen samples enriched for X- or Y-sperm. Methods for increasing the probability that offspring will be male or female comprising the step of allowing as perm from an enriched semen sample to fertilize an ovum. Methods for increasing the probability that offspring will be male or female comprising the step of immunizing a female with X- or Y-SAM proteins. Methods of decreasing fertility comprising the step of immunizing a female with both X- and Y-sperm. Methods of increasing the probability that mammalian offspring will carry a gene for a particular sex-chromosome linked trait.

SUMMARY:

BSUM(13)

Another reason for these failures may be that the head, tail, and plasma membranes of the sperm, its other cellular material, and its highly compact nucleus all act to mask the small DNA content differences between X-sperm and Y-sperm. Some evidence for this masking effect is the fact that cytometric separation, while not feasible for whole sperm, has been useful to prepare enriched subpopulations of denuded sperm nuclei. Using this technique, the sperm nuclei are first separated from the membranes and other material of whole **sperm**. They are then **stained** and partially sorted using a flow cytometer (Johnson and Pinkel, 1986). The result has been nuclei subpopulations enriched for the X- and Y-chromosome.

2. 4,981,686, Jan. 1, 1991, Personal lubricant; Robert E. Hardy, 424/93; 252/52R; 424/430, DIG.14; 514/873; 604/55 [IMAGE AVAILABLE]

US PAT NO: 4,981,686 [IMAGE AVAILABLE] L1: 2 of 28

ABSTRACT:
A personal lubricant which is not contraceptive, does not impede sperm motility and may contain healing ingredients.

DETDESC:

DETD(2)

separation composition contains colloidal silica particles covalently linked to an organosilane having a non-ionic, hydrophilic group.

DETDESC:

DETD(63)

One to six mls of a semen sample containing 1.times.10.sup.7 to 2.times.10.sup.7 sperm/ml is admixed with 6.7 mls diluted reagent-modified colloidal silica which has a density after dilution of 1.185 g/cm.sup.3, a density which is intermediate between the densities of X and Y sperm. The gradient material consists of 1.7 mls of reagent-modified colloidal silica made from colloidal silica of about 70 .ANG. and 5.0 mls of reagent-modified colloidal silica made from colloidal silica of about 200 .ANG.. The admixture is centrifuged at 2,000.times.g for 10 to 15 minutes in a fixed angle rotor. The density of Y sperm is less than X sperm due to the lower DNA content per sperm. The Y sperm are removed using a pipette and cell removal tube. The X sperm are removed using a cell removal tube. The separation is monitored by fluorescence **staining** of the separated **spermatozoa** using acridine orange and quantitated by use of a Fluorescence Activited Cell Sorter (FACS) laser system. The greater DNA content of the X sperm produces a greater fluorescent image relative to the Y sperm. Biol. Reprod. 28: 312-321 (1983).

5. 4,927,749, May 22, 1990, Reagent for cell separation; Allan R. Dorn, 435/2; 424/529, 534, 561; 428/405; 435/261 [IMAGE AVAILABLE]

US PAT NO: 4,927,749 [IMAGE AVAILABLE] L1: 5 of 28

ABSTRACT:
Cell separation compositions and associated methods effective for separating cells from various biological specimens such as blood are disclosed. The biological specimens are contacted with the cell separation compositions and centrifuged. The cells are separated based upon their buoyant density in the cell separation composition. The cell separation composition contains colloidal silica particles covalently linked to an organosilane having a non-ionic, hydrophilic group.

DETDESC:

DETD(62)

One to six mls of a semen sample containing 1.times.10.sup.7 to 2.times.10.sup.7 sperm/ml is admixed with 6.7 mls diluted reagent-modified colloidal silica which has a density after dilution of 1.185 g/cm.sup.3, a density which is intermediate between the densities of X and Y sperm. The gradient material consists of 1.7 mls of reagent-modified colloidal silica made from colloidal silica of about 70 .ANG. and 5.0 mls of reagent-modified colloidal silica made from colloidal silica of about 200 .ANG.. The admixture is centrifuged at 2,000.times.g for 10 to 15 minutes in a fixed angle rotor. The density of Y sperm is less than X sperm due to the lower DNA content per sperm. The Y sperm are removed using a pipette and cell removal tube. The X sperm are removed using a cell removal tube. The separation is monitored by fluorescence **staining** of the separated **spermatozoa** using acridine

A personal lubricant according to the invention includes one or more and preferably a combination of lubricants selected from petrolatum, coconut oil, anhydrous lanolin, mineral oil, egg albumen, and stearly alcohol. In addition to providing desired lubricity, these lubricants are consistent with other characteristics of the invention as being greaseless, non-**staining**, nonspermicidal, nonirritating and not impeding **sperm** motility, and in the case of lanolin being a healing agent. Other preferred ingredients for the lubricant include aloe vera and allantoin as healants, sorbitol as a humectant and for pleasing taste, and almond oil and coconut flavor as fragrances.

3. 4,929,542, May 29, 1990, In vitro screening test for mutagenicity and genotoxicity during spermatogenesis; Michael S. Risley, 435/2, 4, 29, 240.3, 240.31, 243, 244, 809 [IMAGE AVAILABLE]

US PAT NO: 4,929,542 [IMAGE AVAILABLE] L1: 3 of 28

ABSTRACT:
An in vitro screening test for identifying mutagenic and genotoxic agents during spermatogenesis, comprising: (1) Culturing Xenopus testis explants in vitro in the presence of one or more suspected mutagenic and/or genotoxic agent(s); (2) Removing said one or more suspected agent(s) and continue culturing said Xenopus testis explants such that spermatogonia in said Xenopus testis explants undergo spermatogenesis; (3) Isolating sperm and/or one or more types of spermatogenic cells at various times during step (2); and (4) Determining the mutagenic and/or genotoxic affect of said agent(s). A novel medium suitable for culturing Xenopus testis explants. A novel method for culturing Xenopus testis explants in vitro such that cells at all stages of the spermatogenic cycle are produced comprising culturing Xenopus tests fragments in the novel medium under an atmosphere of air and at a temperature in a range of from about 20.degree. C. to about 24.degree. C.

DETDESC:

DETD(112)

Micronuclei occur in round spermatids as a consequence of chromosome damage in pre-spermatid stages, i.e. spermatocytes and spermatogonia, etc. Micronuclei can be detected by fluorescence microscopy of **spermatids** **stained** with the DNA specific fluorochrome, Hoechst 33258. (See, for example, Lahdetie and Parvinen, 1981, Mutat. Res. 81:103-115 Lahdetie, J. 1983, Mutat. Res. 119:79-82 and 120:257-260).

4. 4,927,750, May 22, 1990, Cell separation process; Allan R. Dorn, 435/2; 424/529, 534, 561; 435/243, 261 [IMAGE AVAILABLE]

US PAT NO: 4,927,750 [IMAGE AVAILABLE] L1: 4 of 28

ABSTRACT:

Cell separation compositions and associated methods effective for separating cells from various biological specimens such as blood are disclosed. The biological specimens are contacted with the cell separation compositions and centrifuged. The cells are separated based upon their buoyant density in [redacted] cell separation composition. [redacted] cell

orange and quantitated by use of a Fluorescence Activited Cell Sorter (FACS) laser system. The greater DNA content of the X sperm produces a greater fluorescent image relative to the Y sperm. Biol. Reprod. 28: 312-321 (1983).

6. 4,803,297, Feb. 7, 1989, Carbamic acid ester useful for preparing a nucleic acid probe; Corey H. Levenson, et al., 560/159; 548/303

US PAT NO: 4,803,297 L1: 6 of 28

ABSTRACT:
Nucleic acids may be labeled by intercalating the alkylating intercalation moiety of a labeling reagent into a partially double-stranded nucleic acid to form a complex and activating the complex to cause covalent bonding between the reagent and the nucleic acid. Preferably, the labeled nucleic acid is hybridization probe for detecting nucleic acid sequences capable of hybridizing with a hybridizing region of the nucleic acid. Also preferably the label moiety is non-radioactive. The labeling reagent is of the formula:

[A--[B--L

where A is an alkylating intercalation moiety, B is a divalent organic moiety of the formula: ##STR1## where Y is O, NH or N--CHO, x is a number from 1 to 4, y is a number from 2 to 4, and L is a monovalent label moiety, wherein B is exclusive of any portion of the intercalation and label moieties.
Preferably A is a 4-methylene-substituted psoralen moiety, and most preferably A is a 4'-methylene-substituted-4,5',8-trimethylpsoralen moiety and L is biotin.

DETDESC:

DETD(167)

In the next experiment, the ability of biopsoralenated DNA to hybridize specifically to filter-bound DNA and then be detected by avidin-horseradish peroxidase was tested. For this purpose the three biotin-labeled M13mp9CHL2.1 phage DNAs described above, which contained single-stranded 1.5 kilobase inserts homologous to pCHL2, were hybridized to filters spotted with the Chlamydia trachomatis clone pCHL2 and herring sperm DNA as follows: In this procedure, the plasmid DNA and sperm DNA were applied onto a filter, treated with 0.5N sodium hydroxide for 5 minutes to denature the DNA, and then neutralized by treating with a buffer consisting of 1 M Tris-HCl and 1.5 M NaCl at pH 7. Thereafter, the filter was dried for 2 hours at 80.degree. C. and treated with a prehybridization mixture of 0.2% wt/vol BSA, 0.2% wt/vol Ficoll, 0.2% wt/vol polyvinylpyrrolidone, a buffer of 0.9 M NaCl, 0.05 M sodium phosphate pH 7.0 and 0.005 M EDTA, and 100 .mu.lg/ml of sheared and denatured herring sperm DNA, for about 2 hours. Then the filters were combined with a hybridization solution consisting of 50% wt/vol formamide, 10% wt/vol dextran sulfate, 0.2% wt/vol BSA, 0.2% wt/vol Ficoll, 0.2% wt/vol polyvinylpyrrolidone, a buffer of 0.9 M NaCl. 0.05 M sodium phosphate pH 7.0 and 0.005 M EDTA, 100 .mu.lg/ml of sheared and denatured herring sperm DNA, and about 15 ng/ml of the respective DNA probes. This last hybridization step was carried out in a sealed plastic bag at 42.degree. C. for 20 hc[redacted]. After this time the samples [redacted]e

incubated with the detection solution of avidin and horseradish peroxidase and washed as described above, and then contacted with diaminobenzidine as described above to determine the amount of avidin binding. The results show that the M13 DNA probe labeled with Compound XII stained the darkest, although the probe labeled with Compound II stained almost as darkly. The comparative M13 DNA probe labeled with biotinylated uracil groups **stained** very poorly. The herring **sperm** negative control showed no **stain**. In these experiments the probes of this invention were able to detecting of the target 1.5 kilobase sequence easily.

7. 4,777,133, Oct. 11, 1988, Device for quantitative endpoint determination in immunofluorescence using microfluorophotometry; Grace L. Picciolo, et al., 435/7.22; 250/252.1; 424/3, 7.1; 435/7.36, 29, 34; 436/46, 527, 531, 537, 800, 807, 826

US PAT NO: 4,777,133 L1: 7 of 28

ABSTRACT:
The present invention discloses a device and a process for quantitation of Toxoplasma gondii and Treponema pallidum antibody titer in a biological sample by immunofluorescent photometric microscopy. The process comprises:
(a) reacting a specimen of said sample with an immunofluorescent reagent in a mounting medium containing a protective agent in an amount sufficient to reduce fading of a fluorescent reaction product less than 25% of initial fluorescent intensity;
(b) localizing the specimen under transmitted, visible light;
(c) reducing the effect of counterstain intensity by filters in emission light path;
(d) measuring the sample using a fast shutter;
(e) calibrating the photometer used in said microscopy by a stable fluorophore;
(f) recording intensity of fluorescence of said specimen compared to standard negative and positive controls;
(g) reducing effect of polar staining by substracting the corrected intensity of corresponding dilution of the negative control from the sample reading;
and (h) assigning a numerical endpoint for serum antibody levels against Toxoplasma gondii or Treponema pallidum.

DETDESC:

DETD(53)

Using a XBO 75 W xenon lamp, McKay et al., Immunology 43, 591-602 (1981) measured the fading of conjugates of anti-human gamma globulin-FITC and anti-human gamma globulin-rhodamine B 200 (RB 200). With the RB 200 conjugates, there was little, if any, fading after two min, and this decline could not be separated from instrument error. For fluorescein, however, there was considerable fading which reached a plateau after a certain period of time. This result ws interpreted to mean that fading is the sum of two components, one that decays exponentially and one that remains constant. They subtracted the plateau level value, which represents the non-fading component, from each intensity value and plotted the fading component vs time on semi-logarithmic paper. This plot

produced a straight line which showed the fading obeyed first-order kinetics. They found a half-life of about 1 min for their FITC conjugates. Enerback and Johansson Histochem. J. 5, 351-362 (1973) measured the fading of several fluorochromes including FITC and Feulgen-Schiff using a XBO 75 W xenon lamp and instrumentation capable of recording fluorescence of very short duration. They found a half-life of two 2 seconds for FITC under continuous excitation. For Feulgen-Pararosaniline reaction, there was a 20% loss of initial fluorescence after 20 seconds. They also tested the effect of repeated very short excitation times at two second intervals on the fading. For FITC, there was significant fading after fifteen measurements with illumination times up to 1/60 scond. Using an oscilloscope, they found a fading of 0.5% during the first two 2 ms of illumination. For Feulgen-Schiff stained cells, the fading could be prevented by reducing the illumination time. Bohm and Sprenger Histochemie 16, 100-118 (1968) measured the fading of **sperm** **stained** with several dyes, including acriflavin and Pararosaniline under 5 min continuous excitation using a XBO 150 W xenon lamp. They found a fading rate of 25% and 60%, respectively, for Pararosaniline and Acriflavin.

8. 4,767,703, Aug. 30, 1988, Method for assessing the fertility of male mammals; Roy L. Ax, et al., 435/29, 4, 806; 436/63, 906

US PAT NO: 4,767,703 L1: 8 of 28

ABSTRACT:
A method for evaluating the fertility of a male mammal. A semen sample is obtained from the individual to be tested. The sperm is separated from the seminal plasma of the semen sample. At least one test portion and a control portion of the sperm are isolated. The sperm portions are incubated, the test portion being exposed to a glycosaminoglycan in a concentration effective to induce an acrosome reaction in sperm. A representative sample of incubated sperm from each portion is then counted by means of observation by light microscopy to measure the increase in acrosome reaction in the test portions as compared to the control portion. A kit is provided for the convenient performance of the method. The kit includes a supply of sterile liquid culture medium, sterile closable culture vessels, and a supply of selected, sterile GAG meterable substantially aseptically in known amounts into each culture vessel used as a test vessel.

DETD(7)

Acrosomal staining was accomplished by the method of J. H. D. Bryan and S. R. Akruk (1977), A Naphthol Yellow S and Erythrosin B Staining Procedure for use in Studies of the Acrosome Reaction of Rabbit **Spermatozoa**, **Stain** Technol., 52, 47-50. **Sperm** smears were made on microscope slides to be subjected to the **staining** procedure. A minimum of 100 **sperm** in each slide-mounted sample were counted by light microscopy to determine the percentage of acrosome-reacted sperm.

DETDESC:

DETD(10)

To determine the ability of C[redacted] to induce an acrosome reactio[redacted] the

epididimal bovine spermatozoa described above were incubated for 22 hours in the presence of either 0, 1, 10, or 100 .mu.g/ml of CS-A. The samples were subjected to the acrosomal **staining** procedure referred to above. **Sperm** which accepted the **stain** were considered to have not undergone an acrosome reaction. To confirm the ability of the light microscopy staining method to assay the acrosome reaction, specimens of the spermatozoa before and after exposure to CS-A were examined for vesiculation of the outer acrosomal membrane. The accuracy of the interpretation of staining set forth above was proved to be accurate.

DETDESC:

DETD(15)

The results of incubation with dextran sulfate are also shown in FIG. 2. As can be seen, incubation with dextran sulfate produced no statistically significant effect on the acrosome reaction. The extent of the acrosome reaction in each case was measured by light microscopy, using the **staining** method disclosed above. Once again, **spermatozoa** that had undergone the acrosome reaction were distinguishable from the remaining spermatozoa by their failure to take up stain. As can be seen from the results shown in FIG. 2, each GAG was capable of inducing an acrosome reaction in the rabbit sperm. However, the concentration at which the effect was most pronounced was not the same for each GAG. A comparable experiment was conducted using bovine semen and using the GAG heparan sulfate. The results were substantially the same as those achieved with heparin.

9. 4,764,373, Aug. 16, 1988, Method of increasing the economic value of breeding stock semen; Ronald J. Ericsson, 424/561; 435/2; 604/55

US PAT NO: 4,764,373 L1: 9 of 28

ABSTRACT:
Method of artificially inseminating a plurality of animals with aliquots of sperm obtained from a breeding stock individual of that species, obtained by fractionating the collected semen into first and second motile-sperm containing fractions, the first of which is free from immotile sperm and non-sperm components, both suspended in a liquid vehicle which is physiologically acceptable to the sperm and for artificial insemination; dividing the first and second fractions into a plurality of aliquots, each of which contain enough motile sperm to ensure a pregnancy when used for an artificial insemination; artificially inseminating a plurality of individuals of that species in which a predominance of male offspring is desired with the aliquots of the first fraction; and artificially inseminating a plurality of individuals of that species in which a predominance of male offspring is not a desired objective with the aliquots of the second fraction.

DETDESC:

DETD(8)

The results of this experiment showed that ewes inseminated with spermatozoa from the bottom portion of the BSA column produced more male, and correspondingly fewer female lambs, than did spermatozoa from the top

portion. Thus the Ericsson et al (Ericsson, R. J., Langevin, C. N. and Nishino, M., 1973. Nature, London, 246, 421-424) technique modified for ram spermatozoa, raises the possibility of separating X and Y chromosome bearing spermatozoa populations in the sheep. Unfortunately, it is not possible to identify these two types of sperm in the semen of the ram or other domestic species although the Y chromosome of human **sperm** can be **stained** with quinacrine (Barlow, P. and Vosa, C. G., 1970. Nature, London, 226, 961-962). The additional beneficial effects of this separation procedure observed in other species are increased sperm motility, reduced seminal debris and perhaps reduced sperm with abnormal morphology (Ericsson, R. J. and Glass, R. H., 1982. In Amann, R. P. and Seidel, G. E., Prospects for Sexing Mammalian Sperm, Colorado Association of University Press, Boulder, 201-211).

10. 4,754,065, Jun. 28, 1988, Precursor to nucleic acid probe; Corey H. Levenson, et al., 562/564

US PAT NO: 4,754,065 L1: 10 of 28

ABSTRACT:
Nucleic acids may be labeled by intercalating the alkylating intercalation moiety of a labeling reagent into a partially double-stranded nucleic acid to form a complex and activating the complex to cause covalent bonding between the reagent and the nucleic acid. Preferably, the labeled nucleic acid is a hybridization probe for detecting nucleic acid sequences capable of hybridizing with a hybridizing region of the nucleic acid. Also preferably the label moiety is non-radioactive. The labeling reagent is of the formula:

[A] [B] L
where A is an alkylating intercalation moiety, B is a divalent organic moiety of the formula: ##STR1## where Y is O, NH or N--CHO, x is a number from 1 to 4, y is a number from 2 to 4, and L is a monovalent label moiety, wherein B is exclusive of any portion of the intercalation and label moieties.
Preferably A is a 4-methylene-substituted psoralen moiety, and most preferably A is a 4'-methylene-substituted-4,5',8-trimethylpsoralen moiety and L is biotin.

DETDESC:

DETD(88)

In the next experiment, the ability of biopsoralenated DNA to hybridize specifically to filter-bound DNA and then be detected by avidin-horseradish peroxidase was tested. For this purpose the three biotin-labeled M13mp9CHL2.1 phage DNAs described above, which contained single-stranded 1.5 kilobase inserts homologous to pCHL2, were hybridized to filters spotted with the Chlamydia trachomatis clone pCHL2 and herring sperm DNA as follows: In this procedure, the plasmid DNA and sperm DNA were applied onto a filter, treated with 0.5N sodium hydroxide for 5 minutes to denature the DNA, and then neutralized by treating with a buffer consisting of 1M Tris-HCl and 1.5M NaCl at pH 7. Thereafter, the filter was dried for 2 hours at 80.degree. C. and treated with a prehybridization mixture of 0.2% wt/vol BSA, 0.2% wt/vol Ficoll, 0.2% wt/vol polyvinylpyrrolidone, a[redacted]ffer of 0.9M NaCl, 0.05M sodiu[redacted]

phosphate pH 7.0 and 0.005M EDTA, and 100 .mu.g/ml of sheared and denatured herring sperm DNA, for about 2 hours. Then the filters were combined with a hybridization solution consisting of 50% wt/vol formamide, 10% wt/vol dextran sulfate, 0.2% wt/vol BSA, 0.2% wt/vol Ficoll, 0.2% wt/vol polyvinylpyrrolidone, a buffer of 0.9M NaCl, 0.05M sodium phosphate pH 7.0 and 0.005M EDTA, 100 .mu.g/ml of sheared and denatured herring sperm DNA, and about 15 ng/ml of the respective DNA probes. This last hybridization step was carried out in a sealed plastic bag at 42.degree. C. for 20 hours. After this time the samples were incubated with the detection solution of avidin and horseradish peroxidase and washed as described above, and then contacted with diaminobenzidine as described above to determine the amount of avidin bindings. The results show that the M13 DNA probe labeled with Compound XII stained the darkest, although the probe labeled with Compound II stained almost as darkly. The comparative M13 DNA probe labeled with biotinylated uracil groups **stained** very poorly. The herring **sperm** negative control showed no **stain**. In these experiments the probes of this invention were able to detect 1 ng of the target 1.5 kilobase sequence easily.

11. 4,751,313, Jun. 14, 1988, Precursor to nucleic acid probe; Corey H. Levenson, et al., 548/303

US PAT NO: 4,751,313 L1: 11 of 28

ABSTRACT:
Nucleic acids may be labeled by intercalating the alkylating intercalation moiety of a labeling reagent into a partially double-stranded nucleic acid to form a complex and activating the complex to cause covalent bonding between the reagent and the nucleic acid. Preferably, the labeled nucleic acid is a hybridization probe for detecting nucleic acid sequences capable of hybridizing with a hybridizing region of the nucleic acid. Also preferably the label moiety is non-radioactive. The labeling reagent is of the formula:

[A][B]L

where A is an alkylating intercalation moiety, B is a divalent organic moiety of the formula: ##STR1## where Y is O, NH or N--CHO, x is a number from 1 to 4, y is a number from 2 to 4, and L is a monovalent label moiety, wherein B is exclusive of any portion of the intercalation and label moieties.
Preferably A is a 4-methylene-substituted psoralen moiety, and most preferably A is a 4'-methylene-substituted-4,5',8-trimethylpsoralen moeity and L is biotin.

DETDESC:

DETD(149)

In the next experiment, the ability of biopsoralenated DNA to hybridize specifically to filter-bound DNA and then be detected by avidin-horseradish peroxidase was tested. For this purpose the three biotin-labeled M13mp9CHL2.1 phage DNAs described above, which contained single-stranded 1.5 kilobase inserts homologous to pCHL2, were hybridized to filters spotted with the Chlamydia trachomatis clone pCHL2 and herring sperm DNA as follows: In this procedure, the plasmid DNA and sperm DNA

were applied onto a filter, treated with 0.5N sodium hydroxide for 5 minutes to denature the DNA, and then neutralized by treating with a buffer consisting of 1M Tris-HCl and 1.5 M NaCl at pH 7. Thereafter, the filter was dried for 2 hours at 80.degree. C. and treated with a prehybridization mixture of 0.2% wt/vol BSA, 0.2% wt/vol Ficoll, 0.2% wt/vol polyvinylpyrrolidone, a buffer of 0.9M NaCl, 0.05M sodium phosphate pH 7.0 and 0.005M EDTA, and 100 .mu.g/ml of sheared and denatured herring sperm DNA, for about 2 hours. Then the filters were combined with a hybridization solution consisting of 50% wt/vol formamide, 10% wt/vol dextran sulfate, 0.2% wt/vol BSA, 0.2% wt/vol Ficoll, 0.2% wt/vol polyvinylpyrrolidone, a buffer of 0.9M NaCl, 0.05M sodium phosphate pH 7.0 and 0.005M EDTA, 100 .mu.g/ml of sheared and denatured herring sperm DNA, and about 15 ng/ml of the respective DNA probes. This last hybridization step was carried out in a sealed plastic bag at 42.degree. C. for 20 hours. After this time the samples were incubated with the detection solution of avidin and horseradish peroxidase and washed as described above, and then contacted with diaminobenzidine as described above to determine the amount of avidin binding. The results show that the M13 DNA probe labeled with Compound XII stained the darkest, although the probe labeled with Compound II stained almost as darkly. The comparative M13 DNA probe labeled with biotinylated uracil groups **stained** very poorly. The herring **sperm** negative control showed no **stain**. In these experiments the probes of this invention were able to detect 1 ng of the target 1.5 kilobase sequence easily.

12. 4,741,998, May 3, 1988, Monoclonal antibody to MHS-5: a new probe for sexual assault analyses; John C. Herr, et al., 435/7.92, 28, 70.21, 172.2, 240.27, 810, 948, 975; 436/518, 543, 547, 548, 808; 530/387; 935/104, 108, 110

US PAT NO: 4,741,998 L1: 12 of 28

ABSTRACT:
A new probe for forensic analysis of sexual assaults is disclosed. The probe is a monoclonal antibody to a new protein marker, MHS-5, in semen. Also disclosed is the hybridoma producing the antibody as well as an assay utilizing the antibody for forensic analysis of criminal evidence.

DRAWING DESC:

DRWD(2)

FIG. 1 shows human **sperm** before and after being **stained** with the MHS-5 (FIG. B) monoclonal antibody and polyclonal antibody (FIG. D). The differential interference contrast images of the corresponding fluorescent sperm are presented in A and C.

DETDESC:

DETD(10)

Incubation with the monoclonal antibody to MHS-5 antigen resulted in intense staining of the post, acrosomal, midpiece and tail regions of the **sperm** with lesser **staining** of the acrosome (FIG. B). To carry out the method, 1.times.10.sup.5 s[redacted]m were added to wells of eight[redacted]ll

microslides and then air dried. Sperm were bathed with one percent bovine serum albumin in phosphate buffered saline for 30 minutes. Slides were washed three times in phosphate buffered saline. Ascites fluid containing the monoclonal antibody to MHS-5 anitgen was diluted 1:50 and then added to each well for 30 minutes. The slides were then wased five times in phosphate buffered saline. Fluorescein isothiocyanate--goat anti-mouse immunoglobulin at 1:150 dilution was added to the slides and incubated for 45 minutes. The slides were then washed three times with phosphate buffered saline and then mounted with a drop of glycerol. The photography was performed with a light microsocpe (Leitz) equipped with a 100x-13.2NA flotar objective.

13. 4,705,886, Nov. 10, 1987, Precursor to nucleic acid probe; Corey H. Levenson, et al., 560/159; 548/303; 562/564; 930/10, 220

US PAT NO: 4,705,886 L1: 13 of 28

ABSTRACT:
Nucleic acids may be labeled by intercalating the alkylating intercalation moiety of a labeling reagent into a partially double-stranded nucleic acid to form a complex and activating the complex to cause covalent bonding between the reagent and the nucleic acid. Preferably, the labeled nucleic acid is a hybridization probe for detecting nucleic acid sequences capable of hybridizing with a hybridizing region of the nucleic acid. Also preferably the label moiety is non-radioactive. The labeling reagent is of the formula:

[A--[B--L
where A is an alkylating intercalation moiety, B is a divalent organic moiety of the formula: ##STR1## where Y is O, NH or N--CHO, x is a number from 1 to 4, y is a number from 2 to 4, and L is a monovalent label moiety, wherein B is exclusive of any portion of the intercalation and label moieties.
Preferably A is a 4-methylene-substituted psoralen moiety, and most preferably A is a 4'-methylene-substituted-4,5', 8-trimethylpsoralen moiety and L is biotin.

This patent application is a divisional application of copending U.S. Ser. No. 791,332 filed Oct. 25, 1985, now U.S. Pat. No. 4,617,261, which is a continuation-in-part application (CIP) of copending U.S. Ser. No. 683,263 filed Dec. 18, 1984, now U.S. Pat. No. 4,582,789 which is a CIP of copending U.S. Ser. No. 591,811 filed Mar. 21, 1984, now abandoned. This patent application is also related to copending U.S. application Ser. No. 791,323 filed Oct. 25, 1985.

DETDESC:

DETD(146)

In the next experiment, the ability of biopsoralenated DNA to hybridize specifically to filter-bound DNA and then be detected by avidin-horseradish perioxidase was tested. For this purpose the three biotin-labeled M13mp9CHL2.1 phage DNAs described above, which contained single-stranded 1.5 kilobase inserts homologous to pCHL2, were hybridized to filters spotted with the Chalmydia trachomatis clone pCHL2 and herring sperm DNA as follows: In this procedure, the plasmid DNA and sperm DNA

were applied onto a filter, treated with 0.5N sodium hydroxide for 5 minutes to denature the DNA, and then neutralized by treating with a buffer consisting of 1M Tris-HCl and 1.5M NaCl at pH 7. Thereafter, the filter was dried for 2 hours at 80.degree. C. and treated with a prehybridization mixture of 0.2% wt/vol BSA, 0.2% wt/vol Ficoll, 0.2% wt/vol polyvinylpyrrolidone, a buffer of 0.9M NaCl, 0.05M sodium phosphate pH 7.0 and 0.005M EDTA, and 100 .mu.g/ml of sheared and denatued herring sperm DNA, for about 2 hours. Then the filters were combined with hybridization solution consisting of 50% wt/vol formamide, 10% wt/vol dextran sulfate, 0.2% wt/vol BSA, 0.2% wt/vol Ficoll, 0.2% wt/vol polyvinylpyrrolidone, a buffer of 0.9M NaCl, 0.05M sodium phosphate pH 7.0 and 0.005M EDTA, 100 .mu.g/ml of sheared and denatured herring sperm DNA, and about 15 ng/ml of the respective DNA probes. This last hybridization step was carried out in a sealed plastic bag at 42.degree. C. for 20 hours. After this time the samples were incubated with the detection solution of avidin and horseradish perioxidase and washed as described above, and then contacted with diaminobenzidine as described above to determine the amount of avidin binding. The results show that the M13 DNA probe labeled with Compound XII stained the darkest, although the probe labled with Compound II stained almost as darkly. The comparative M13 DNA probe labeled with biotinylated uracil groups **stained** very poorly. The herring **sperm** negative control showed no **stain**. In these experiments the prbes of this invention were able to detect 1 ng of the target 1.5 kilobase sequence easily.

14. 4,643,967, Feb. 17, 1987, Antibody method for lowering risk of susceptibility to HLA-associated diseases in future human generations; Bernard J. Bryant, 435/7.21, 960; 436/543, 821

US PAT NO: 4,643,967 L1: 14 of 28

ABSTRACT:
A method and apparatus for reducing the risk of occurrence in human offspring of diseases in which the susceptibility of the individual is known to be linked to the gene complex encoding the human leukocyte antigens (HLA). Antibody molecules specific for an HLA antigen having a known disease association are used to deplete native semen samples with respect to spermatozoa bearing the targeted HLA antigen on their surface membranes while leaving unaffected, and suitable for fertilization, other spermatozoa in the sample which lack the disease-associated HLA antigen. The specific depletion is effected by cellular killing or inhibition of motility through linkage of the HLA antibody bound by spermatozoa to a cytotoxic molecule such as complement.

DETDESC:

DETD(14)

Applicant has applied the steps and procedures delineated above in phase one upon many occasions and experience reveals specific FA staining in approximately one-half of all sperms after exposure to antisera to either HLA antigens A1 or A3 but not A2 or A9, see Table 2. The HLA antigens expressed on an individual mature sperm theoretically can reflect either its own genetic composition (haploid gene expression) or that of its diploid precursor cell. Haploid expression in the present instance predicts that the sperm popula[redacted]n as a whole will be divided e[redacted]lly

into subpopulations of A1 positive and A3 positive cells. This is verified by the results of FA staining using A1 or A3 antisera as shown in Table 2. This interpretation is further verified by the additive effect (nearly 100% FA **staining** of **sperms**) observed when A1 and A3 antisera were used together, see Table 2. The few FA positive sperms observed after exposure to A2 or A9 antisera probably express the known presence of extraneous HLA antibodies in the commercial tissue typing antisera used. Thus the results of the methods and steps employed in phase one of the instant invention verify haploid expression, and tissue types the semen donor as positive for HLA A-locus antigens A1 and A3. After phase one has been completed and the semen sample has been verified for haploid expression and particular tissue typing, then the remainder of the sample can be treated before artificial insemination according to the steps of phase two delineated hereinbelow.

15. RE 32,350, Feb. 10, 1987, Thermal convection counter streaming sedimentation and forced convection galvanization method for controlling the sex of mammalian offspring; Bhairab C. Bhattacharya, 204/180.1, 299R

US PAT NO: RE 32,350 L1: 15 of 28

ABSTRACT:
A method and apparatus for controlling the sex of mammalian offspring by separation of the X-chromosome female producing sperm and Y-chromosome male producing sperm according to their different characteristics of density of the respective cells and electric potential on the respective cell surfaces. Separation is accomplished by first producing at thermal convection counter stream within a sedimentation column containing a universal medium with sperm suspended therein and allowing the two sperm populations to settle into different fractions according to different densities. Subsequently, the fractions are further separated and concentrated utilizing convection galvanization. The positive and negative geotaxis applied to the sperm during thermal convection sedimentation in combination with galvanic forces applied during the convection galvanization facilitate a more efficient separation than previously obtained. This is due to the fact that a greater degree of separation of X and Y sperm is achieved by subjecting an unbalanced population of sperm cells, i.e., one predominating in X or Y cells, to convection galvanization. Thermal convection counter streaming sedimentation has been found to be a preferred method for attaining this unbalanced sperm population. The apparatus used to accomplish the above separation includes means for producing a temperature differential between axial and peripheral portions of the medium contained in the sedimentation column, thus creating the necessary thermal convection counter stream, as well as an electrophoreses cell comprising a convection column disposed between the two electrodes of the cell. Alternatively, the sedimentation apparatus and the convection galvanization apparatus may be combined. Additionally, the apparatus may comprise a laser capable of scanning the length of the thermal convection sedimentation column as well as laser detecting means to determine the distribution of sperm produced within the medium therein.

DETDESC:

DETD(27)

Alternatively to the above, the purity of the separated and/or non-separated semen may be checked with the B-body test. This test is based on the knowledge that Y spermatozoa of humans and primates tends to fluoresce with a special brightness when stained with quinacrine-HCL or quinacrine-mustard with the staining technique being simple and generally accepted by the prior art. While attempts have been made in the past to extend this technique in order to identify male and female spermatozoa in other domestic animals besides humans and primates, failure has been reported in many instances. My analysis on the subject encouraged me to develop a successful technique of **staining** Y **spermatozoa** by this method in other species since human and primate sperm contain a large quantity of proteolotic enzymes which partially dissolve the sialic acid-protein complex, glyco- and lypo-protein coating of the sperm membrane, which has been found to enhance the penetration of the dye into the chromatin materials. Thus, after the utilization of different enzymes at different concentrations, temperatures, and pH's, etc., I have particularly found that papaya protease (available Sigma Chemicals, U.S.A.) is suitable for the purpose of performing a similar function artificially on the cell membranes of the sperm of other domestic animals.

DETDESC:

DETD(28)

The above process is carried out as follows: Approximately 1 milliliter of semen or medium mixture (containing from about 20 million to 50 million cells) is washed with saline and may be centrifuged three times at 2,500 grams for 15 minutes. This centrifuging and washing, if it is in addition to that first described above, may be optional. After diluting the sediment with 1 milliliter of fresh saline in the present example, 3 drops of this suspension is mixed with 5 milligrams of protease and allowed to digest for approximately 10 minutes at room temperature. Subsequently, one drop of 0.005% quinacrine-mustard is added to 1 drop of the digested mixture, put on a slide, and mounted immediately for microscopic examination. After allowing 40 minutes for the dye to enter the inner structure of the spermatozoa, which is facilitated by the digestion of the outer membrane by protease, the spermatozoa may be viewed with a Leitz Ortholux microscope using the KP-490 Eciter Filter at a transmission wave length of 530 namometers with two heat barriers. HP430 and HP 460. Human spermatozoa treated in the above matter may take a deep **stain** without identifying Y chromosome containing **sperm**; however, bull and horse spermatozoa with the Y chromosome show a distinct bright spot with excellent visibility (B-body) with the X bearing **spermatozoa** taking a dull and diffused **stain**. Nonprocessed bull, human and horse semen and processed bull semen have been checked for B-bodies as a routine procedure for checking product purity in the practice of the present invention. The results in 523 experiments are tabulated below in Table I where the results from B-body tests are compared with that from biological tests.

CLAIMS:

CLMS(10)

10. A method as recited in cl[redacted] 1 further comprising the step[redacted]f:

staining any Y chromosome containing **sperm** cells present in said withdrawn fraction of medium with a compound selected from the group consisting of quinacrine HCl and quinacrine-mustard; and

determining the proportion of sperm cells contained in said withdrawn fraction of medium which are **stained** relative to those **sperm** cells which are not.

CLAIMS:

CLMS(11)

11. A method as recited in claim 10 further comprising the step of treating sperm cells contained in said withdrawn fraction of medium with an enzyme capable of enhancing the penetration of said **staining** compound into the **sperm** cells.

16. 4,622,291, Nov. 11, 1986, Method and device for quantitative end point determination in immunofluorescence using microfluorophotometry; Grace L. Picciolo, et al., 435/4; 250/252.1; 424/3, 7.1; 435/29; 436/46, 527, 531, 537, 800, 807, 826

US PAT NO: 4,622,291 L1: 16 of 28

ABSTRACT:
The present invention discloses a device and a process for quantitation of end point in the formation of fluorescent reaction product in microfluorophotometry. The process comprises:

(a) incorporating a protective agent in a suitable mounting medium in an amount sufficient to reduce fading of fluorescent reaction product less than 25% of initial fluorescent intensity;

(b) calibrating photometer used in said microscopy with a stable emitter; and

(c) recording the intensity of fluorescence of said fluorescent reaction product by means for measuring light intensity.

The invention also includes a device for calibration of the photometer and a kit comprising separate containers for suitable mounting medium, buffer, suitable immunofluorescent reagents, fading retardant means, a photometer calibrating device and the like and optional instructions.

DETDESC:

DETD(53)

Using a XBO 75 W xenon lamp, McKay et al., Immunology 43, 591-602 (1981) measured the fading of conjugates of anti-human gamma globulin-FITC and anti-human gamma globulin-rhodamine B 200 (RB 200). With the RB 200 conjugates, there was little, if any, fading after two min, and this decline could not be separated from instrument error. For fluorescein, however, there was considerable fading which reached a plateau after a certain period of time. This result was interpreted to mean that fading is the sum of two components, one that decays exponentially and one that remains constant. They subtracted the plateau level value, which represents the non-fading component, from each intensity value and plotted the fading component vs time on semi-logarithmic paper. This plot produced a straight line which showed the fading obeyed first-order kinetics. They found a half-life of about 1 min for their FITC

conjugates. Enerback and Johansson Histochem. J. 5, 351-362 (1973) measured the fading of several fluorochromes including FITC and Feulgen-Schiff using a XBO 75 W xenon lamp and instrumentation capable of recording fluorescence of very short duration. They found a half-life of two 2 seconds for FITC under continuous excitation. For Feulgen-Pararosaniline reaction, there was a 20% loss of initial fluorescence after 20 seconds. They also tested the effect of repeated very short excitation times at two second intervals on the fading. For FITC, there was significant fading after fifteen measurements with illumination times up to 1/60 second. Using an oscilloscope, they found a fading of 0.5% during the first two 2 ms of illumination. For Feulgen-Schiff stained cells, the fading could be prevented by reducing the illumination time. Bohm and Sprenger Histochemie 16, 100-118 (1968) measured the fading of **sperm** **stained** with several dyes, including Acriflavin and Pararosaniline under 5 min continuous excitation using a XBO 150 W xenon lamp. They found a fading rate of 25% and 60%, respectively, for Pararosaniline and Acriflavin.

17. 4,617,261, Oct. 14, 1986, Process for labeling nucleic acids and hybridization probes; Edward L. Sheldon, III, et al., 435/6, 7.24, 7.5, 7.9; 436/94, 501; 548/303; 930/220; 935/78

US PAT NO: 4,617,261 L1: 17 of 28

ABSTRACT:
Nucleic acids may be labeled by intercalating the alkylating intercalation moiety of a labeling reagent into a partially double-stranded nucleic acid to form a complex and activating the complex to cause covalent bonding between the reagent and the nucleic acid. Preferably, the labeled nucleic acid is a hybridization probe for detecting nucleic acid sequences capable of hybridizing with a hybridizing region of the nucleic acid. Also preferably the label moiety is non-radioactive. The labeling reagent is of the formula:

[A--[B--L

where A is an alkylating intercalation moiety, B is a divalent organic moiety of the formula: ##STR1## where Y is O, NH or N--CHO, x is a number from 1 to 4, y is a number from 2 to 4, and L is a monovalent label moiety, wherein B is exclusive of any portion of the intercalation and label moieties.
Preferably A is a 4-methylene-substituted psoralen moiety, and most preferably A is a 4'-methylene-substituted-4,5',8-trimethylpsoralen moeity and L is biotin.

DETDESC:

DETD(146)

In the next experiment, the ability of biopsoralenated DNA to hybridize specifically to filter-bound DNA and then be detected by avidin-horseradish peroxidase was tested. For this purpose the three biotin-labeled M13mp9CHL2.1 phage DNAs described above, which contained single-stranded 1.5 kilobase inserts homologous to pCHL2, were hybridized to filters spotted with the Chlamydia trachomatis clone pCHL2 and herring sperm DNA as follows: In this procedure, the plasmid DNA and sperm DNA were applied onto a filter, treated with 0.5N sodium hydroxide for 5

minutes to denature the DNA, and then neutralized by treating with a buffer consisting of 1M Tris-HCl and 1.5M NaCl at pH 7. Thereafter, the filter was dried for 2 hours at 80.degree. C. and treated with a prehybridization mixture of 0.2% wt/vol BSA, 0.2% wt/vol Ficoll, 0.2% wt/vol polyvinylpyrrolidone, a buffer of 0.9M NaCl, 0.05M sodium phosphate pH 7.0 and 0.005M EDTA, and 100 .mu.g/ml of sheared and denatured herring sperm DNA, for about 2 hours. Then the filters were combined with a hybridization solution consisting of 50% wt/vol formamide, 10% wt/vol dextran sulfate, 0.2% wt/vol BSA, 0.2% wt/vol Ficoll, 0.2% wt/vol polyvinylpyrrolidone, a buffer of 0.9M NaCl, 0.05M sodium phosphate pH 7.0 and 0.005M EDTA, 100 .mu.g/ml of sheared and denatured herring sperm DNA, and about 15 ng/ml of the respective DNA probes. This last hybridization step was carried out in a sealed plastic bag at 42.degree. C. for 20 hours. After this time the samples were incubated with the detection solution of avidin and horseradish peroxidase and washed as described above, and then contacted with diaminobenzidine as described above to determine the amount of avidin binding. The results show that the M13 DNA probe labeled with Compound XII stained the darkest, although the probe labeled with Compound II stained almost as darkly. The comparative M13 DNA probe labeled with biotinylated uracil groups **stained** very poorly. The herring **sperm** negative control showed no **stain**. In these experiments the probes of this invention were able to detect 1 ng of the target 1.5 kilobase sequence easily.

18. 4,582,789, Apr. 15, 1986, Process for labeling nucleic acids using psoralen derivatives; Edward L. Sheldon, III, et al., 435/6, 7.5, 7.9; 436/501; 930/10; 935/77, 78

US PAT NO: 4,582,789 L1: 18 of 28

ABSTRACT:
A labeling reagent of the formula:

[A][B]L

is prepared where A is an alkylating intercalation moiety, B is a divalent organic spacer arm moiety with a straight chain of at least two carbon atoms, and L is a monovalent label moiety capable of producing a detectable signal, e.g., a signal detectable by spectroscopic, photochemical, chemical, immunochemical or biochemical means. Preferably A is a 4'-methylene-substituted psoralen moiety, and most preferably A is a 4'-methylene-substituted 4,5',8-trimethylpsoralen moiety.
This reagent may be used to label nucleic acids, preferably DNA, by intercalating the alkylating intercalation moiety of the reagent into an at least partially double-stranded nucleic acid to form a complex and activating the complex to cause covalent bonding between the reagent and the nucleic acid. Preferably, the labeled nucleic acid is a hybridization probe for detecting nucleic acid sequences capable of hybridizing with a hydridizing region of the nucleic acid. Also preferably the label moiety is non-radioactive.
This reagent may also be used in chromosome banding to label specific regions of chromosomes and thereby differentiate them.

DETDESC:

DETD(124)

In the next experiment, the ability of biopsoralenated DNA to hybridize specifically to filter-bound DNA and then be detected by avidin-horseradish peroxidase was tested. For this purpose the three biotin-labeled M13mp9CHL2.1 phage DNAs described above, which contained single-stranded 1.5 kilobase inserts homologous to pCHL2, were hybridized to filters spotted with the Chlamydia trachomatis clone pCHL2 and herring sperm DNA as follows: In this procedure, the plasmid DNA and sperm DNA were applied onto a filter, treated with 0.5N sodium hydroxide for 5 minutes to denature the DNA, and then neutralized by treating with a buffer consisting of 1M Tris--HCl and 1.5M NaCl at pH 7. Thereafter, the filter was dried for 2 hours at 80.degree. C. and treated with a prehybridization mixture of 0.2% wt/vol BSA, 0.2% wt/vol Ficoll, 0.2% wt/vol polyvinylpyrrolidone, a buffer of 0.9M NaCl, 0.05M sodium phosphate pH 7.0 and 0.005M EDTA, and 100 .mu.g/ml of sheared and denatured herring sperm DNA, for about 2 hours. Then the filters were combined with a hybridization solution consisting of 50% wt/vol formamide, 10% wt/vol dextran sulfate, 0.2% wt/vol BSA, 0.2% wt/vol Ficoll, 0.2% wt/vol polyvinylpyrrolidone, a buffer of 0.9M NaCl, 0.05M sodium phosphate pH 7.0 and 0.005M EDTA, 100 .mu.g/ml of sheared and denaturated herring sperm DNA, and about 15 ng/ml of the respective DNA probes. This last hybridization step was carried out in a sealed plastic bag at 42.degree. C. for 20 hours. After this time the samples were incubated with the detection solution of avidin and horseradish peroxidase and washed as described above, and then contacted with diaminobenzidine as described above to determine the amount of avidin binding. The results show that the M13 DNA probe labeled with Compound XII stained the darkest, although the probe labeled with Compound II stained almost as darkly. The comparative M13 DNA probe labeled with biotinylated uracil groups **stained** very poorly. The herring **sperm** negative control showed no **stain**. In these experiments the probes of this invention were able to detect 1 ng of the target 1.5 kilobase sequence easily.

19. 4,559,309, Dec. 17, 1985, Flow cytometry-fluorescence measurements for characterizing sperm; Donald P. Evenson, et al., 436/63; 250/461.2; 436/94, 172

US PAT NO: 4,559,309 L1: 19 of 28

ABSTRACT:
A process for characterizing **sperm** motility and viability by **staining** a **sperm** sample with Rhodamine 123 and ethidium bromide, and simultaneously measuring the sperm fluorescence emissions at green frequencies 515-575 nm and at red frequencies 600-650 nm, the green counts being correlated with sperm motility and the red counts being correlated with putative dying or dead cells. Additionally, a sample of sperm can be characterized as to type and normality by **staining** a sample of **sperm** with acridine orange and simultaneously measuring the sperm fluorescence emissions at green frequencies 515-575 nm and at red frequencies 600-650 nm.

ABSTRACT:
A process for characterizing **sperm** motility and viability by **staining** a **sperm** sample with Rhodamine 123 and ethidium bromide, and simultaneously measuring t[redacted] sperm fluorescence emissions a[redacted]reen

frequencies 515-575 nm and at red frequencies 600-650 nm, the green counts being correlated with sperm motility and the red counts being correlated with putative dying or dead cells. Additionally, a sample of sperm can be characterized as to type and normality by **staining** a sample of **sperm** with acridine orange and simultaneously measuring the sperm fluorescence emissions at green frequencies 515-575 nm and at red frequencies 600-650 nm.

SUMMARY:

BSUM(11)

Following a similar procedure **sperm** samples are also **stained** with Rhodamine 123 (Red) and ethidium bromide (green) and measured with flow cytometry techniques giving a measure of mitochondrial membrane potential which correlates with cell motility and cell viability.

DRAWING DESC:

DRWD(3)

FIG. 1 shows a two parameter histogram demonstrating **stainability** of human **sperm** cells with rhodamine 123 and ethidium bromide.

DETDESC:

DETD(3)

Flow cytometry of **sperm** cells **stained** green with Rhodamine 123 (R123) and red with ethidium bromide (EB) provides a rapid, quantitative analysis of mitochondrial membrane potential (correlated here with cell motility) and cell viability. Staining green or red with acridine orange and applying flow cytometry measuring techniques results in measurements which can be correlated with (a) the percentage of each cell type in semen including, (i) mature sperm, (ii) immature sperm precursor cells representing all stages of development from spermatogonia to mature sperm, (iii) somatic cells e.g. leukocytes; (b) normality/abnormality of sperm nuclear chromatin condensation.

DETDESC:

DETD(5)

The **sperm** in suspension, **stained** with AO, R 123 and EB, were measured individually in a flow cytometer at rates up to one thousand per second. The resulting data permits full characterization of sperm, accomplished quickly and easily. Thus, statistically significant populations were easily assayed, offering an advantage over the slower, semi-quantitative methods used currently for analyses of sperm motility and viability. Also the use of mitochondrial membrane potential as a mesurement of cell motility was not previously reported and the use of AO for cell morphology indexing is more sensitive than current light microscope techniques thereby giving more and/or better information.

DETDESC:

DETD(11)

Sperm in suspension, **stained** with R123/EB as described above were observed by U.V. and light microscopy using a Leitz Orthoplan microscope fitted with epifluorescent illumination (485 nm excitation and 530 nm emission filters). The green fluorescence of R123 was restricted to the midpiece containing the mitochondria, and the red fluorescence of EB staining putative dying or dead cells was located in the sperm head. Bright green fluorescence correlated with fast motility. Bright red and green fluorescence were mutually exclusive. However, some cells did exhibit both pale red and pale green fluorescence localized in the head and midpiece respectively. These and other data indicated that impaired or dying cells stained with R123 under non-equilibrium conditions lose green fluorescence before, or as they begin to show red fluorescence. Somatic cells undergo a transient increase in uptake of R123 prior to cell death and lysis, Darzynkiewicz et al, Cancer Research, 42:799 not seen in dying sperm cells.

DETDESC:

DETD(31)

The relationship between increased levels of F.sub.530 **staining** in **sperm** and chromatin structure is not clear as yet. Current electron microscope studies (Evenson, to be published) suggest that the chromatin is not as condensed in **sperm** with increased F.sub.530 **staining** as in normal, fertile controls. The relationship between chromatin condensation and fertility is unclear also since it is not known whether sperm with less condensed chromatin are on average less likely to successfully fertilize ova. In this context, however, other studies in this laboratory have shown a relationship between chromatin structure and fertility, i.e. sperm nuclear DNA is less resistant to thermal denaturation in situ in cases of subfertility. Current work also suggests that **sperm** demonstrating increased F.sub.530 **stainability** have increased thermal denaturation of the DNA in situ. However, thermal denaturation is the more sensitive technique for detection of alterations in chromatin structure that may be related to fertility. Cases of questionable fertility in humans and bulls have been observed despite normal sperm count, motility index and morphology by both classical light microscope methods and FCM analysis of AO stained semen aliquots; however, following thermal stress of the isolated nuclei (7) the DNA denatures easily indicating an abnormality of chromatin structure.

CLAIMS:

CLMS(1)

What is claimed is:

1. Process for characterizing sperm motility and viability comprising the steps of

staining a sample of the **sperm** with Rhodamine 123 and ethidium bromide,

applying flow cytographic measuring techniques to count, simultaneously, the sperm fluorescence emissions at green frequencies 515-575 nm and at red frequencies 600-650 nm, [redacted] green counts being correlated [redacted]th

sperm motility and the red counts being correlated with putative dying or dead cells.

CLAIMS:

CLMS(2)

2. Process of claim 1 further comprising **staining** a second sample of the **sperm** with acridine orange and applying flow cytographic measuring techniques to count, simultaneously, the sperm fluorescence emissions at green frequencies 515-575 nm and at red frequencies 600-650 nm, which counts are correlated with cell type and normality.

20. 4,448,767, May 15, 1984, Preparation of monospecific male-specific antibody and the use thereof for increasing the percentage of mammalian offspring of either sex; Bernard J. Bryant, 424/85.9, 561; 435/2

US PAT NO: 4,448,767 L1: 20 of 28

ABSTRACT:
A method of manufacturing and a raw material and its useage are disclosed for increasing the percentage of mammalian offspring of either sex. The process of use utilizes a male-specific antibody coupled to a solid-phase immunoabsorbent material to effect a separation of sex-determining spermatozoa derived from a semen suspension. The male-specific antibody selectively binds male-determining spermatozoa, while the female-determining spermatozoa remain not bound and are recoverable. A male specific antibody that is monospecific is prepared from male cells subjected to a multistep process which includes using said cells for the hyperimmunization of a female species and the preparation of an antiserum which is purified as to specificity by a multiplicity of absorptions against female cells. The antiserum is fractionated to yield male specific monospecific immunoglobulin which may be used to effect a separation of sex determining spermatozoa.

DETDESC:

DETD(46)

If the male-specific antibody is frozen prior to its usage for coupling to an immunoabsorbent in accordance with another portion of my invention, there could be some loss of specificity if more than one freeze-thaw cycle has occurred during storage. To verify specificity, one may conduct the specificity test previously discussed or another similar test to be set forth currently. The monospecificity of the male-specific antiserum may be tested by coupling a portion of it with fluorescein isothyiocyanate. In carrying out this test, the fluorescent-tagged antiserum is combined with human spermatozoa in a direct fluorescense microscopic preparation. Bright fluorescence of approximately 50 percent of the sperm indicate specific binding of the fluorescent tagged male-specific antiserum by Y **sperm**. The lack of **staining** of the remaining approximately 50 percent of the sperm, i.e. the X sperm, indicates the essential monospecificity of the anti-serum produced byd antiserum monospecificity test, if it were shown in FIG. 2, would immediately follow the agarose gel chromatography indicated in the step

of reference numeral 18.

21. 4,397,954, Aug. 9, 1983, Flowcell fractionator; Siddhartha Sarkar, 435/287; 209/158; 435/2

US PAT NO: 4,397,954 L1: 21 of 28

ABSTRACT:
Enriched quantities of male and female sperm are obtained in physically separate fractions utilizing the hydrodynamic behavior of sperm in laminar flow. A flowcell fractionator is provided for performing the method, and includes a specially constructed pipette, valve and infusion pump combination.

DETDESC:

DETD(33)

Isolation of X chromosome bearing sperm may be achieved by combining mechanical and biochemical selective techniques. For example, in connection with the utilization of the apparatus of FIG. 1, sperm motility can be made conditionally dependent upon the utilization of hypoxanthine by the X-linked enzyme activity, EC 2.4.2.8. hypoxanthine phosphoribosyl transferase (HPRT), when the de novo pathway of purine synthesis is inhibited with aminopterin. Under this selection condition, the Y chromosome positive sperm loose their motility faster than the Y chromosome negative cells, thereby allowing one to enrich for X chromosome bearing sperm in the motile fraction obtained with the apparatus of FIG. 1. Immunofluorescense **staining** of **sperm** HPRT shows that the enzyme has a broad quantitive variation among sperm populations unlike the all or none distribution of sex chromosomes.

22. 4,360,013, Nov. 23, 1982, Polymeric acid contraceptive devices; Thomas H. Barrows, 128/832; 424/DIG.14; 604/55

US PAT NO: 4,360,013 L1: 22 of 28

ABSTRACT:
A disposable non-woven sponge vaginal contraceptive device is made of alginic acid or related polysaccharides bearing carboxylic acid functionality and a method of making same is disclosed.

DETDESC:

DETD(27)

Although the acidity of alginic acid is an important factor in causing sperm death, this factor is apparently not the only mechanism of activity. Rabbit semen was acidified with physiological saline solution containing hydrochloric acid and cell death determined by use of a "live-dead" **stain**. The percent of dead **sperm** that were also decapitated--indicating severe cell disruption--was also recorded. The results of this test at various pH values were compared to the results obtained by contacting rabbit semen with the alginic acid-polyester blend sponge materials from the samples described above. The results, shown in TABLE II, clearly indicate tha[redacted]lginic acid is unexpectedly mo[redacted]potent

as a spermicide than anticipated on the basis of its acid strength.

23. 4,326,026, Apr. 20, 1982, Method for fractionating cells; Siddhartha Sarkar, 435/2; 424/561; 435/240.1, 240.2

US PAT NO: 4,326,026 L1: 23 of 28

ABSTRACT:
Enriched quantities of male and female sperm are obtained in physically separate fractions utilizing the hydrodynamic behavior of sperm in laminar flow. A flowcell fractionator is provided for performing the method, and includes a specially constructed pipette, valve and infusion pump combination.

DETDESC:

DETD(33)

Isolation of X chromosome bearing sperm may be achieved by combining mechanical and biochemical selective techniques. For example, in connection with the utilization of the apparatus of FIG. 1, sperm motility can be made conditionally dependant upon the utilization of hypoxanthine by the X-linked enzyme activity, EC 2.4.2.8. hypoxanthine phosphoribosyl transferase (HPRT), when the de novo pathway of purine synthesis is inhibited with aminopterin. Under this selection condition, the Y chromosome positive sperm loose their motility faster than the Y chromsome negative cells, thereby allowing one to enrich for X chromsome bearing sperm in the motile fraction obtained with the apparatus of FIG. 1. Immunofluorescense **staining** of **sperm** HPRT shows that the enzyme has a broad quantitive variation among sperm populations unlike the all or none distribution of sex chromosomes.

24. 4,191,749, Mar. 4, 1980, Method and material for increasing the percentage of mammalian offspring of either sex; Bernard J. Bryant, 424/561, 85.9, 88; 435/2

US PAT NO: 4,191,749 L1: 24 of 28

ABSTRACT:
Method, apparatus and material are disclosed for increasing the percentage of mammalian offspring of either sex. The method utilizes a male-specific antibody coupled to a solid-phase immunoabsorbent material to effect a separation of sex-determining spermatazoa derived from a semen suspension. The male-specific antibody selectively binds male-determining spermatazoa. The female-determining spermatazoa are not bound and are recovered directly from the male-specific antibody-coated immunoabsorbent material. The male-determining spermatazoa are recovered from the male-specific antibody-coated immunoabsorbent material after altering the condition thereof to inhibit binding. The apparatus includes a vertical surface comprising beads of immunoabsorbent material over which are distributed male-specific antibodies. The materials are two seminal fluids, one having a substantial preponderance of male-determining spermatazoa, and the other having a substantial preponderance of female-determining spermatazoa.

DETDESC:

DETD(10)

The monospecificity of the male-specific antiserum obtained by the just described technique may be tested by coupling a portion of it with fluorescein isothiocyanate. In carrying through this test of the present invention the fluorscent-tagged antiserum is combined with human spermatazoa in a direct fluoresence microscopic preparation. Bright fluorescence of approximately 50 percent of the sperm indicate specific binding of the fluorescent tagged male-specific antiserum by Y **sperm**. The lack of **staining** of the remaining approximately 50 percent of the sperm, i.e., the X sperm, indicates the essential monospecificity of the antiserum produced by the just described technique for the Y antigen.

25. 4,185,085, Jan. 22, 1980, Differential diagnostic sperm examination; Carsten A. Carstensen, 424/3; 8/506; 424/7.1; 435/2

US PAT NO: 4,185,085 L1: 25 of 28

ABSTRACT:
A method of differential diagnostic sperm examination and the use of prestained slides in that method comprising placing the specimen to be examined on a prestained blood-picture slide, developing the stain and examining same thereafter.

SUMMARY:

BSUM(15)

It has now been found, quite unexpectedly, that the morphological properties of sperms can be shown in an outstanding manner when one drop of ejaculate is placed on prestained blood-picture slides, as described, for example, in U.S. Pat. No. 4,070,495, capped with a cover glass, and examined microscopically after the **stain** has developed. The **sperms** then show up in a high-contrast coloration while the background remains colorless.

SUMMARY:

BSUM(20)

In the case of the cells of the spermiogenesis (spermatogonia, **spermatocytes** and **spermides**), the nuclei are **stained** dark purple and the plasma light violet, as with the spermatozoa, so that they are readily distinguished from the leukocytes on the basis of their morphology.

26. 4,155,831, May 22, 1979, Thermal convection counter streaming sedimentation and forced convection galvanization method and apparatus for controlling the sex of mammalian offspring; Bhairab C. Bhattacharya, 204/299R, 180.1

US PAT NO: 4,155,831 L1: 26 of 28

ABSTRACT:
A method and apparatus for con[redacted]lling the sex of mammalian off[redacted]ing by

separation of the X-chromosome female producing sperm and Y-chromosome male producing sperm according to their different characteristics of density of the respective cells and electric potential on the respective cell surfaces. Separation is accomplished by first producing a thermal convection counter stream within a sedimentation column containing a universal medium with sperm suspended therein and allowing the two sperm populations to settle into different fractions according to different densities. Subsequently, the fractions are further separated and concentrated utilizing convection galvanization. The positive and negative geotaxis applied to the sperm during thermal convection sedimentation in combination with galvanic forces applied during the convection galvanization facilitate a more efficient separation than previously obtained. This is due to the fact that a greater degree of separation of X and Y sperm is achieved by subjecting an unbalanced population of sperm cells, i.e., one predominating in X or Y cells, to convection galvanization. Thermal convection counter streaming sedimentation has been found to be a preferred method for attaining this unbalanced sperm population. The apparatus used to accomplish the above separation includes means for producing a temperature differential between axial and peripheral portions of the medium contained in the sedimentation column, thus creating the necessary thermal convection counter stream, as well as an electrophoreses cell comprising a convection column disposed between the two electrodes of the cell. Alternatively, the sedimentation apparatus and the convection galvanization apparatus may be combined. Additionally, the apparatus may comprise a laser capable of scanning the length of the thermal convection sedimentation column as well as laser detecting means to determine the distribution of sperm produced within the medium therein.

DETDESC:

DETD(27)

Alternatively to the above, the purity of the separated and/or non-separated semen may be checked with the B-body test. This test is based on the knowledge that Y spermatozoa of humans and primates tends to fluoresce with a special brightness when stained with quinacrine-HCL or quinacrine-mustard with the staining technique being simple and generally accepted by the prior art. While attempts have been made in the past to extend this technique in order to identify male and female spermatozoa in other domestic animals besides humans and primates, failure has been reported in many instances. My analysis on the subject encouraged me to develop a successful technique of **staining** Y **spermatozoa** by this method in other species since human and primate sperm contain a large quantity of proteolotic enzymes which partially dissolve the sialic acid-protein complex, glyco- and lypo-protein coating of the sperm membrane, which has been found to enhance the penetration of the dye into the chromatin materials. Thus, after the utilization of different enzymes at different concentrations, temperatures, and pH's, etc., I have particularly found that papaya protease (available Sigma Chemicals, U.S.A.) is suitable for the purpose of performing a similar function artificially on the cell membranes of the sperm of other domestic animals.

DETDESC:

DETD(28)

The above process is carried out as follows: Approximately 1 milliliter of semen or medium mixture (containing from about 20 million to 50 million cells) is washed with saline and may be centrifuged three times at 2,500 grams for 15 minutes. This centrifuging and washing, if it is in addition to that first described above, may be optional. After diluting the sediment with 1 milliliter of fresh saline in the present example, 3 drops of this suspension is mixed with 5 miligrams of protease and allowed to digest for approximately 10 minutes at room temperature. Subsequently, one drop of 0.005% quinacrine-mustard is added to 1 drop of the digested mixture, put on a slide, and mounted immediately for microscopic examination. After allowing 40 minutes for the dye to enter the inner structure of the spermatozoa, which is facilitated by the digestion of the outer membrane by protease, the spermatozoa may be viewed with a Leitz Ortholux microscope using the KP-490 Eciter Filter at a transmission wave length of 530 nanometers with two heat barriers, HP 430 and HP 460. Human spermatozoa treated in the above manner may take a deep **stain** without identifying Y chromosome containing **sperm**; however, bull and horse spermatozoa with the Y chromosome show a distinct bright spot with excellent visibility (B-body) with the X bearing **spermatozoa** taking a dull and diffused **stain**. Nonprocessed bull, human and horse semen and processed bull semen have been checked for B-bodies as a routine procedure for checking product purity in the practice of the present invention. The results in 523 experiments are tabulated below in Table I where the results from B-body tests are compared with that from biological tests.

27. 4,092,229, May 30, 1978, Thermal convection counter streaming sedimentation and forced convection galvanization method for controlling the sex of mammalian offspring; Bhairab C. Bhattacharya, 204/180.1, 299R; 209/11, 173; 435/2, 3

US PAT NO: 4,092,229 L1: 27 of 28

ABSTRACT:
A method and apparatus for controlling the sex of mammalian offspring by separation of the X-chromosome female producing sperm and Y-chromosome male producing sperm according to their different characteristics of density of the respective cells and electric potential on the respective cell surfaces. Separation is accomplished by first producing a thermal convection counter stream within a sedimentation column containing a universal medium with sperm suspended therein and allowing the two sperm populations to settle into different fractions according to different densities. Subsequently, the fractions are further separated and concentrated utilizing convection galvanization. The positive and negative geotaxis applied to the sperm during thermal convection sedimentation in combination with galvanic forces applied during the convection galvanization facilitate a more efficient separation than previously obtained. This is due to the fact that a greater degree of separation of X and Y sperm is achieved by subjecting an unbalanced population of sperm cells, i.e., one predominating in X or Y cells, to convection galvanization. Thermal convection counter streaming sedimentation has been found to be a preferred method for attaining this unbalanced sperm population. The apparatus used to accomplish the above separation includes means for [redacted]ducing a temperature different[redacted]

between axial and peripheral portions of the medium contained in the sedimentation column, thus creating the necessary thermal convection counter stream, as well as an electrophoreses cell comprising a convection column disposed between the two electrodes of the cell. Alternatively, the sedimentation apparatus and the convection galvanization apparatus may be combined. Additionally, the apparatus may comprise a laser capable of scanning the length of the thermal convection sedimentation column as well as laser detecting means to determine the distribution of sperm produced within the medium therein.

DETDESC:

DETD(27)

Alternatively to the above, the purity of the separated and/or non-separated semen may be checked with the B-body test. This test is based on the knowledge that Y spermatozoa of humans and primates tends to fluoresce with a special brightness when stained with quinacrine-HCL or quinacrine-mustard with the staining technique being simple and generally accepted by the prior art. While attempts have been made in the past to extend this technique in order to identify male and female spermatozoa in other domestic animals besides humans and primates, failure has been reported in many instances. My analysis on the subject encouraged me to develop a successful technique of **staining** Y **spermatozoa** by this method in other species since human and primate sperm contain a large quantity of proteolotic enzymes which partially dissolve the sialic acid-protein complex, glyco- and lypo-protein coating of the sperm membrane, which has been found to enhance the penetration of the dye into the chromatin materials. Thus, after the utilization of different enzymes at different concentrations, temperatures, and pH's, etc., I have particularly found that papaya protease (available Sigma Chemicals, U.S.A.) is suitable for the purpose of performing a similar function artificially on the cell membranes of the sperm of other domestic animals.

DETDESC:

DETD(28)

The above process is carried out as follows: Approximately 1 milliliter of semen or medium mixture (containing from about 20 million to 50 million cells) is washed with saline and may be centrifuged three times at 2,500 grams for 15 minutes. This centrifuging and washing, if it is in addition to that first described above, may be optional. After diluting the sediment with 1 milliliter of fresh saline in the present example, 3 drops of this suspension is mixed with 5 miligrams of protease and allowed to digest for approximately 10 minutes at room temperature. Subsequently, one drop of 0.005% quinacrine-mustard is added to 1 drop of the digested mixture, put on a slide, and mounted immediately for microscopic examination. After allowing 40 minutes for the dye to enter the inner structure of the spermatozoa, which is facilitated by the digestion of the outer membrane by protease, the spermatozoa may be viewed with a Leitz Ortholux microscope using the KP-490 Eciter Filter at a transmission wave length of 530 nanometers with two heat barriers, HP 430 and HP 460. Human spermatozoa treated in the above manner may take a deep **stain** without identifying Y chromosome containing **sperm**;

however, bull and horse spermatozoa with the Y chromosome show a distinct bright spot with excellent visibility (B-body) with the X bearing **spermatozoa** taking a dull and diffused **stain**. Nonprocessed bull, human and horse semen and processed bull semen have been checked for B-bodies as a routine procedure for checking product purity in the practice of the present invention. The results in 523 experiments are tabulated below in Table I where the results from B-body tests are compared with that from biological tests.

CLAIMS:

CLMS(10)

10. A method as recited in claim 1 further comprising the steps of: **staining** any Y chromosome containing **sperm** cells present in said withdrawn fraction of medium with a compound selected from the group consisting of quinacrine HCl and quinacrine-mustard; and determining the proportion of sperm cells contained in said withdrawn fraction of medium which are **stained** relative to those **sperm** cells which are not.

CLAIMS:

CLMS(11)

11. A method as recited in claim 10 further comprising the step of treating sperm cells contained in said withdrawn fraction of medium with an enzyme capable of enhancing the penetration of said **staining** compound into the **sperm** cells.

28. 4,083,957, Apr. 11, 1978, Process for the alteration of the sex-ratio of mammals; John L. Lang, 424/78, 79, 80, 81, 82, 83, 561

US PAT NO: 4,083,957 L1: 28 of 28

ABSTRACT:
The invention provides an improved method for alteration of the sex ratio in animal (including human) offspring by separation of the population of spermatozoa into fractions which are different by reason of the sex-linked electrical charge resident thereon. The separation is carried out by bringing the spermatozoa into close association with an electrostatic charge-bearing material having a charge the sign of which is opposite to the sign of a chosen portion of the spermatozoa which carries the sex determining character of the unwanted sex, so as to attract and thereby to permit that portion to be isolated, or put to a disadvantage in the fertilization of ova. The invention is concerned with the selection of the charge-bearing material, the adjustment of the pH and particle size thereof, and the control of the surrounding medium in relation to its influence on the charge characteristics of both the charge-bearing material and the spermatozoa.

SUMMARY:

BSUM(14)

Other observations confirm th[redacted]xistance of a charge of opposi[redacted]sign on

the spermatozoa of the respective sexes. Thus, dye-**staining** of human **spermatozoa** with quinacrine produces a yellow spot on the "F-body" of the male spermatozoa. The curious part about this is that the Y (male) chromosome itself does not stain with quinacrine because both quinacrine and the Y chromosome are positively charged, whereas the negatively charged X (female) chromosome does stain with quinacrine. This indicates that an electrostatic reversal of sign takes place between the inside of the spermatozoa and the surface of the cell membrane. I am not certain why this takes place, but it points to the existence of complex relationships between the proteinaceous substance of the cell membrane and its surrounding medium which control the nature of the charge. On the other hand, the fact that there is a selective dye staining between the respective sexes supports a conclusion that there are different charge characteristics between the sexes.

DETDESC:

DETD(163)

In another test, human **spermatozoa** was "**stained**" at a pH of 7.2 with a positively charged treating agent which was a microgel of a quaternized copolymer of vinyl benzyl chloride with 0.3 weight % of divinyl benzene. Ultra-violet fluoresence microscopy at about 970X showed that the positively charged microgel was attached to approximately half of the living spermatozoa, in a manner which appears visually to be identical to the manner in which quinacrine concentrates on the F-body in the quinacrine **staining** technique of male **spermatozoa** identification.
=>

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FILE 'BIOSIS' ENTERED AT 13:13:15 ON 30 AUG 91
L1 572 STAIN?(5A)SPERM?
L2 18 L1 AND TEMP?
L3 2 (STAIN?(5A)TEMPERAT?)(5A)SPERM?
L4 2 (STAIN?(5A)SPERM?)(5A)TEMPERAT?
L5 0 L4 NOT L3
L6 3 (STAIN?(5A)SPERM?)(10A)TEMPERAT?
L7 1 L6 NOT L4
L8 56 L1 AND DEGREE?
L9 25 L1 AND (3#(3A)DEGREE#)

=> d 1-25 bib ab

L9 ANSWER 1 OF 25

AN 91:301987 BIOSIS
DN BA92:23002
TI EFFECT OF TEMPERATURE OF THAWING AND DILUENT ON THE POST-THAW PHYSIOLOGICAL CHANGES OF BUFFALO FROZEN SEMEN.
AU DUDEJA S
CS DEP. BIOCHEM., ALL INDIA INST. MED. SCI., NEW DELHI - 110 029.
SO INDIAN J PHYSIOL PHARMACOL 34 (4). 1990. 267-270. CODEN: IJPPAZ ISSN: 0019-5499
LA English
AB The effect of thawing was studied in buffalo semen diluted in three diluents (Tris egg-yolk, Egg-yolk citrate and Citric Acid whey) at three temperatures (5. ***degree*** . C, ***35*** . ***degree*** . C and 75.degree. C) on motility, eosin staining, morphological and acrosomal changes, hyaluronidase, glutamic oxalacetic transaminase and glutamic pyruvic transaminase activities. The motility and lack of ***staining*** of ***sperm*** by eosin were maximum on thawing at ***35*** . ***degree*** . C and in tris egg-yolk diluent followed by egg-yolk citrate and citric acid whey. Hyaluronidase, glutamic oxalacetic transaminase and glutamic pyruvic transaminase increased significantly in the extra-cellular fluid on thawing of semen diluted with all the three diluents. The buffalo semen diluted in tris egg-yolk and thawed at ***35*** . ***degree*** . C for ***30*** seconds rk~
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JULY, 1980 TO DATE.

CHEMICAL NAMES (CNs) ADDED FROM JANUARY 1980 TO DATE.

RECORDS LAST ADDED: 27 August 91 (910827/ED) BA9206 BR4105
CAS REGISTRY NUMBERS (R) LAST ADDED: 28 August 91 (910828/UP)

Changes to SUPERTERM/BC searching -- See HELP STERMS

=> (stain?(5a)sperm?) and (3#(3a)degree#)

82602 STAIN?
54191 SPERM?
572 STAIN?(5A)SPERM?

1429288 3#
277184 DEGREE#

70903 3#(3A)DEGREE#
L1 25 (STAIN?(5A)SPERM?) AND (3#(3A)DEGREE#)

=> d 1-25 bib ab

L1 ANSWER 1 OF 25

AN 91:301987 BIOSIS
DN BA92:23002
TI EFFECT OF TEMPERATURE OF THAWING AND DILUENT ON THE POST-THAW PHYSIOLOGICAL CHANGES OF BUFFALO FROZEN SEMEN.
AU DUDEJA S
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SO INDIAN J PHYSIOL PHARMACOL 34 (4). 1990. 267-270. CODEN: IJPPAZ ISSN: 0019-5499
LA English
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morphological and acrosomal changes, hyaluronidase, glutamic oxalacetic transaminase and glutamic pyruvic transaminase activities. The motility and lack of ***staining*** of ***sperm*** by eosin were maximum on thawing at ***35*** . ***degree*** . C and in tris egg-yolk diluent followed by egg-yolk citrate and citric acid whey. Hyaluronidase, glutamic oxalacetic transaminase and glutamic pyruvic transaminase increased significantly in the extra-cellular fluid on thawing of semen diluted with all the three diluents. The buffalo semen diluted in tris egg-yolk and thawed at ***35*** . ***degree*** . C for ***30*** seconds gave the best results.

L1 ANSWER 2 OF 25

AN 91:222977 BIOSIS
DN BA91:114437
TI CRYOPRESERVATION ACTIN LOCALIZATION AND THERMOTROPIC PHASE TRANSITIONS IN RAM SPERMATOZOA.
AU HOLT W V; NORTH R D
CS INST. ZOOL., ZOOLOGICAL SOCIETY LONDON, REGENT'S PARK, LONDON NW1 4RY, UK.
SO J REPROD FERTIL 91 (2). 1991. 451-462. CODEN: JRPFA4 ISSN: 0022-4251
LA English
AB The effects of controlled stress, i.e. cooling, upon the distribution of actin in ram spermatozoa were examined to investigate the hypothesis that cytoskeletal proteins are involved in the maintenance of sperm plasma membrane integrity. The normal distribution of actin on the spermatozoon was initially determined. A monoclonal antibody (IgM) interacted exclusively with the post-acrosomal region and the principal piece of the flagellum. By the use of a polyclonal antibody, actin was detected on the acrosome (excluding the equatorial segment), the post-acrosomal region and the whole of the flagellum. The actin was present in its non-filamentous form. Spermatozoa fixed at ***39*** ***degree*** C and then treated for the immunofluorescent detection of actin with the monoclonal antibody were mostly unstained (proportion stained=4.4% (.+-. 1.cntdot.6; s.e.m.); n=8 ejaculates). Provided spermatozoa were permeabilized by >0.025% Triton X-100 before immunofluorescence, actin was localized in the post-acrosomal region of all sperm heads, and to a minor extent on the prinicpal piece of the flagellum. Use of the polyclonal antibody confirmed that the post-acrosomal antigen was unmasked by detergent treatment. Slow cooling, over 2-h periods to various temperatures between 5 and 15.degree. C, also induced an increase in the proportion of cells showing post-acrosomal actin immunoreactivity. Cooling through the temperature range 15 to 10.degree. C markedly increased the proportion of immunoreactive cells (mean .+-. s.e.m.; 12 .+-. 4.5% at 15.degree. C; 27 .+-. 4.5% at 10.degree. C; n=4 ejaculates). Further cooling to 5.degree. C failed to elicit increases ***staining*** . Ultrastructural examination of cooled ***spermatozoa*** confirmed that a subpopulation of spermatozoa exhibited post-acrosomal actin immunoreactivity after cooling. These results are compatible with the suggestion that actin fulfils a stabilizing function in spermatozoa.

L1 ANSWER 3 OF 25

AN 91:76142 BIOSIS

DN BA91:44802
TI CRYOPRESERVATION OF BOAR SEMEN IN MINI AND MAXI-STRAWS.
AU BWANGA C O; DE BRAGANCA M M; EINARSSON S; RODRIGUEZ-MARTINEZ H
CS DEP. CLIN. STUDIES, FAC. VET. MED., UNIV. NAIROBI, P.O. BOX 29053, NAIROBI, KENYA.
SO J VET MED SER A 37 (9). 1990. 651-659. CODEN: JVMAE6 ISSN: 0931-184X
LA English
AB Split ejaculates from four boars were frozen with a programmable freezing machine, in mini-(025 ml) and maxi- (5 ml) plastic straws with an extender at either acidic (6.3) or alkaline (7.4) pH. Glycerol (3%) was used as cryoprotectant. The freezing of the semen was monitored by way of thermocouples placed in the straws. Post-thaw motility and acrosome integrity were evaluated; the latter using phase contrast microscopy, eosin-nigrosin ***stain*** and electron microscopy. Post-thaw ***sperm*** mortility was significantly higher when semen was frozen in mini-straws than in maxi-straws. For the mini-straws, the motility was better when semen was exposed to an acidic environment during freezing, but this beneficial effect of the low extracellular pH was not evident when maxi-straws were thawed. The motility of the spermatozoa diminished significantly during the thermoresistance test (0h and 2 h time) at ***37*** . ***degree*** . C in a similar way for both straws and extracellular pH's. The freezing procedure, no matter the extracellular pH, did not cause major acrosomal damages, but significantly more normal apical ridges were present in the mini-straws than in the maxi-straws. This in vitro evaluation indicated that the freezing method employed was better for mini- than for maxi-straws since the freezing of the 5 ml volumes was not homogeneous, due to the large section area between the surface and the core of the straw.

L1 ANSWER 4 OF 25

AN 91:44990 BIOSIS
DN BA91:23271
TI EFFECTS OF PLATELET ACTIVATING FACTOR ON THE MOTILITY AND ACROSOME REACTION OF BOVINE SPERMATOZOA.
AU PARKS J E; HOUGH S R
CS 201 MORRISON HALL, CORNELL UNIV., ITHACA, N.Y. 14853.
SO THERIOGENOLOGY 34 (5). 1990. 903-912. CODEN: THGNBO ISSN: 0093-691X
LA English
AB The effects of platelet activating factor (PAF) on motility and the acrosome reaction of ejaculated bull spermatozoa were evaluated. Washed spermatozoa (30 .times. 106/ml) were incubated (***39*** . ***degree*** . C) for up to 2 h with 10 to 200 .mu.M PAF in a modified Tyrode's solution (pH 7.4) containing 3 mg/ml bovine serum albumin. Sperm motility was evaluated subjectively and by computer assisted semen analysis. Percent acrosome-reacted spermatozoa was quantified microscopically from fixed smears following Giemsa ***staining*** . Percent fertilization by PAF-treated ***spermatozoa*** was determined using in vitro-matured bovine ova. Percent sperm motility decreased with .gtoreq. 50 .mu.M PAF, while the rate of motility loss increased with PAF concentration ($P < 0.001$). Percent acrosome reactions increased with PAF concentration during incubation ($P < 0.001$). Acrosomal loss was rapid and complete with 200 .mu.M PAF. At con[redacted]trations between 80 to 120 .mu[redacted]PAF,

bull spermatozoa underwent acrosome reactions without a rapid loss of motility and penetrated in vitro-matured bovine ova at a rate comparable to that of heparincapacitated spermatozoa (68 versus 54%, respectively). Incubation of bull spermatozoa with 10 to 50 .mu.M PAF for 45 min had no effect on percent progressive motility, sperm velocity or other motility parameters. These results indicate that PAF can be used to induce acrosome reactions in bull spermatozoa and to promote in vitro fertilization of bovine ova. Under the conditions used in this study, PAF did not stimulate bovine sperm motility.

L1 ANSWER 5 OF 25

AN 91:321 BIOSIS
DN BA91:321
TI A SIMPLE TECHNIQUE FOR THE INDUCTION AND DETECTION OF THE ACROSOME REACTION IN BULL SPERMATOZOA.
AU KUSUNOKI H; KATO S; KANDA S
CS GRADUATE SCH. SCI. TECHNOL., KOBE UNIV., NADA-KU, KOBE-SHI 657.
SO JPN J ZOOTECH SCI 61 (9). 1990. 831-836. CODEN: NICKA3 ISSN: 0021-5309
LA English
AB Cryopreserved bull spermatozoa were thawed, washed, resuspended at high concentration in K-3 medium, which contained NaCl, CaCl2 and NaHCO3, and preincubated in sealed glass tubes at ***39*** .5. ***degree*** .C. After preincubation, trypan blue-Giemsa (TG) staining and the hamster test were used to identify acrosome-reacted ***spermatozoa*** . TG ***stained*** smears revealed four types of ***spermatozoon*** with different ***staining*** patterns. Since a significant positive correlation was obtained only between the percentage of spermatozoa with both unstained or light-blue postacrosomal regions and unstained or partially reddish-purple acrosomal regions and the percentage of zona-free hamster eggs penetrated ($r = 0.732$, $P < 0.05$, $n = 12$), such spermatozoa were considered to be acrosome-reacted. In bulls A and B, significantly high percentages of acrosome-reacted spermatozoa (A; 37.0%, B; 43.8 - 49.2%) and rates of egg penetration (A; 91.7%, B; 85.4 - 97.7%) were obtained with samples preincubated in K-3 medium for 2 h and 1-2 h, respectively. These results may indicate that bull spermatozoa can undergo the acrosome reaction when they are incubated in a simple medium that contains only NaCl, CaCl2 and NaHCO3 in a sealed glass tube at ***39*** .5. ***degree*** .C and that the TG method can be used for the identification of acrosome-reacted cells.

L1 ANSWER 6 OF 25

AN 90:500434 BIOSIS
DN BA90:128780
TI GLYCOSAMINOGLYCAN SULFATE AS PLASMA MEMBRANE COMPONENT OF PIG SPERMATOZOA.
AU DELGADO N M; REYES R; CARRANCO A; HUACUJA L; MERCHANT H; ROSADO A
CS DIV. BIOL. DESARROLLO, UNIDAD INVESTIGACION BIOMEDICA OCCIDENTE, I.M.S.S., APARTADO POSTAL 2-332, GUADALAJARA, JAL. MEX.
SO ARCH ANDROL 25 (2). 1990. 121-130. CODEN: ARANDR ISSN: 0148-5016
LA English
AB The effect of specific glycosaminoglycan-hydrolyzing enzymes on the ruthenium red ***staining*** of pig ***spermatozoa*** was

studied. Washed spermatozoa were incubated at ***35*** . ***degree*** . C in buffer or with neuraminidase 0.5 units/ml, heparinase 0.2 mg/ml, or chondroitinase ABC 2.0 units/ml. After incubation ***sperm*** cells were washed, ***stained*** with ruthenium red and studied under the electron microscope. Anionic sites in the surface of untreated spermatozoa follow regularly the plasma membrane, but present are numerous processes constituting what has been defined as the glycocalyx. Neuraminidase did not affect the distribution of ruthenium red on the surface of the spermatozoa, but eliminated almost completely the processes of the glycocalyx. Heparinase caused loss of the ruthenium red-stained sites on the membrane surface of pig spermatozoa with less influence on the dense processes of the glycocalyx. A similar loss of ruthenium red-stained sites was observed with nitrous acid treatment. A striking effect of treatment with chondroitinase ABC was the production of a typical acrosome reaction.

L1 ANSWER 7 OF 25

AN 90:374118 BIOSIS
DN BA90:60799
TI PRELIMINARY STUDY ON THE CRYOPRESERVATION OF PACIFIC OYSTER OOCYTES.
AU CHEN C-P; HSU H-W; LEI S-F; CHANG C-H
CS INST. ZOOL., ACAD. SINICA, NANKANG, TAIPEI, TAIWAN 11529.
SO J FISH SOC TAIWAN 16 (3). 1989. 197-202. CODEN: TSCKD6 ISSN: 0379-4180
LA English
AB Oocytes of the Pacific oyster, Crassostrea gigas were obtained from the ovaries of dissected oysters, incubated with cyroprotectants for 10 min at 20.degree. C and then stained with 0.5% trypan blue to investigate the toxicity of the cryoprotectants. Twenty per cent (V/V) of DMSO (dimethyl sulfoxide), propylene and glycerol caused 15,41 and 68% respectively of mortality of the oocyte. Oyster oocytes were incubated with 10% DMSO for 10 min, forzen from 20.degree. C to -14.degree. C at a rate of 5.degree. C/min, then to - ***30*** . ***degree*** . C (0.5.degree. C/min), holding for 5 min, quickly to -150. ***degree*** . C (***35*** . ***degree*** . C/min) and stored in LN2. After thawing with ***37*** . ***degree*** . C water bath, oocytes were fertilized with fresh ***sperm*** and ***stained*** with trypan blue 10 min later. The survival ratio of oocytes stored in LN2 for 10 min, 1 hr and 4 days was 0.69.+-.0.05 (n=2)0.51 .+-. 0.07 (n=2) and 0.10.+-.0.04 (n=3), respectively.

L1 ANSWER 8 OF 25

AN 89:378630 BIOSIS
DN BA88:59220
TI IDENTIFYING THE FLUORESCENT BODY F-BODY WITH QUINACRINE MUSTARD STAINING OF CANINE.
AU IGARASHI Y; TAKESHIMA T; TAUCHI K; TANAKA K; OGAWA S
CS IMAMICHI INST. ANIM. REPRODUCTION, 1103, FUKAYA, DEJIMA-MURA, NIIHARI-GUN, IBARAKI, 300-10, JPN.
SO EXP ANIM (TOKYO) 38 (3). 1989. 263-268. CODEN: JIDOAA ISSN: 0007-5124
LA Japanese
AB The fluorescent body (F-bo[redacted] was identifyed with quinacrin[redacted]ustard

(Q-M) ***staining*** in ***spermatozoon*** and lymphocyte of canine. Well washed sperm suspension was treated with protease (125mg/ml) or dispase (2000p. u./ml) and staining with Q-M (final dilution 50 .mu.g/ml) for 15 min to 24 hr at ***37*** . ***degree*** .C. The lymphocyte cultures from whole blood were prepared as rutine human investigation. The chromosomal preparation made by air dry method was stained with Q-M (final dilution 0.5 to 50 .mu.g/ml) after pretreatment of enzyme digestation. The examination using a reflected fluorescent microscope revealed that the same F-body in human was present in both spermatozoon (20, 1-39. 7%) and interphase of lymphocyte (0-37.2%) of male origin.

L1 ANSWER 9 OF 25

AN 89:295370 BIOSIS
DN BA88:20714
TI ASSESSMENT OF THE VIABILITY AND FERTILIZING POTENTIAL OF CRYOPRESERVED BOVINE ***SPERMATOZOA*** USING DUAL FLUORESCENT ***STAINING*** AND TWO-FLOW CYTOMETRIC SYSTEMS.
AU ERICSSON S A; GARNER D L; REDELMAN D; AHMAD K
CS DEP. ANIM. SCI., UNIV. NEVADA-RENO, RENO, NEVADA 89557-0104.
SO GAMETE RES 22 (4). 1989. 355-368. CODEN: GAMRDC ISSN: 0148-7280
LA English
AB A dual fluorescent staining system utilizing 5 (and-6)-carboxy-4',5'-dimethyl fluorescein diacetate (CDMFDA) and Hydroethidine (HED) was developed to provide quantifiable information reflective of spermatozoal viability and fertilizing potential. Cryopreserved spermatozoa from ten bulls on which there was fertilizing capacity information were incubated for 1.5, and ***3*** hr at ***39*** . ***degree*** . C prior to fluorogenic ***staining*** . ***Spermatozoa*** were analyzed using both a FACS Analyzer and an EPICS V flow cytometer to determine if a particular fluorescence pattern was due to an instrumental artifact or cellular processes. Five fluorescent cellular populations were identified by the FACS Analyzer and three populations by the EPICS V. Spermatozoa were quantified after each incubation time for red (HED) and green (CDMFDA) fluorescence. Viable spermatozoa retained the greatest amount of both green and red fluorescence. Dead or moribund spermatozoa had a decrease in over-all fluorescence. The number of viable cells at 0 hr plus the number of dead or morbid cells at any time period were identified by the FACS Analyzer as important in estimating the potential fertility of a bull. The EPICS V identified the number of dead or moribund cells as being related to nonreturn rates. Incubation of samples decreased cellular viability, which resulted in reduced levels of both green and red fluorescence. Similarities between data obtained with both flow cytometers illustrated that cellular processes, not instrumental artifacts, were responsible for the decrease in over-all fluorescence when viability declined, the relationship between the number of cells with specific fluorescence levels and nonreturn rates, and the incubative-induced changes in fluorescence patterns.

L1 ANSWER 10 OF 25

AN 89:120214 BIOSIS
DN BA87:54867

TI STAINING PROCEDURE TO DETECT VIABILITY AND THE TRUE ACROSOME REACTION IN SPERMATOZOA OF VARIOUS SPECIES.
AU DIDION B A; DOBRINSKY J R; GILES J R; GRAVES C N
CS DEP. ANIM. SCI., 315 ANIMAL SCI. LAB., 1207 WEST GREGORY, URBANA, ILL. 61801.
SO GAMETE RES 22 (1). 1989. 51-58. CODEN: GAMRDC ISSN: 0148-7280
LA English
AB A simple dual ***stain*** procedure (DS) for simultaneously determining ***sperm*** viability and acrosomal status is described. The DS includes the use of the vital stain trypan blue to detect live and dead spermatozoa and Giemsa to detect the presence or absence of an acrosome. For ***staining*** , ***spermatozoa*** are washed, incubated with trypan blue, washed, dried onto slides and subjected to Giemsa. Dead ***spermatozoa*** ***stain*** blue in the postacrosomal region while live ***spermatozoa*** remain unstained. The acrosome ***stains*** light purple-dark pink while acrosome-free ***sperm*** remain unstained. This ***staining*** pattern enables differentiation of ***spermatozoa*** which have undergone a true acrosome reaction (TAR) from those which have undergone a false acrosome reaction (FAR). Incubation of bull, boar, ram, and stallion spermatozoa for 60 minutes at ***37*** . ***degree*** . C in the presence of calcium ionophore A23187 increased the proportion of spermatozoa undergoing a TAR in all species except the stallion. Incubation of bull spermatozoa for up to 24 hours at ***37*** . ***degree*** . C resulted in a decrease over time in the percentage of live acrosome-intact spermatozoa and a simultaneous increase in the percentage of spermatozoa categorized as having undergone a TAR and FAR. The DS could be a useful technique in evaluating sperm viability and acrosomal status in fertilization and clinical studies.

L1 ANSWER 11 OF 25

AN 89:78429 BIOSIS
DN BA87:42827
TI EVIDENCE FOR SEQUENTIAL DEPLOYMENT OF SECRETORY ENZYMES DURING THE NORMAL ACROSOME REACTION OF GUINEA-PIG SPERM IN-VITRO.
AU DICARLANTONIO G; TALBOT P
CS DEP. BIOL., UNIV. CALIF., RIVERSIDE, CALIF. 92521.
SO GAMETE RES 21 (4). 1988. 425-438. CODEN: GAMRDC ISSN: 0148-7280
LA English
AB Experiments were conducted to determine if acrosomal enzymes are released simultaneously or in sequence during the normal acrosome reaction. Epididymal guinea pig sperm were incubated in a chemically defined, calcium-containing medium which supports normal acrosome reactions within 4-5 hours at ***37*** . ***degree*** . C. The sperm suspensions were monitored for motility, normal acrosome reactions, and false acrosome reactions during in vitro incubation. At specified time intervals, the sperm were separated from the incubation medium by centrifugation, and the distribution of dipeptidyl peptidase (DPP II) and acrosin activity was determined by biochemically assaying the hydrolysis of trialanine and N-benzoyl-L-arginine ethyl ester (BAEE), respectively. When calcium was present, there was a significant increase in DPP II activity in the supernatants by 1 hour of incubation and a slight decline at later time points. This re[redacted]se was not correlated with fal[redacted]or

normal acrosome reactions (loss of the acrosomal cap) monitored by phase-contrast microscopy but probably represents a very early stage in the normal acrosome reaction. This early stage is difficult to detect at the light microscope level because sperm are still in rouleaux and because membrane fusion is not directly observable. In contrast, acrosin activity, which was assayed in the same supernatants, increased at later times when sperm were observed to have completed normal acrosome reactions. The ultrastructural distribution of DPP II was determined in sperm pellets collected during in vitro incubation by using the DPP II substrate lysyl-alanyl-4-methoxy-2-naphthyamide. In freshly isolated cauda epididymal sperm, reaction product is confined to the light-staining area in the dorsal bulge of the acrosome. However, by 1 hour of incubation, the light- ***staining*** area of many ***sperm*** was partially or completely dispersed, while other regions of the acrosome were unchanged. Our data are consistent with the conclusions that DPP II is a highly soluble component of the guinea pig sperm acrosome and that its release occurs during the initial phase of the acrosome reaction while sperm are still in rouleaux. Structural changes in the acrosome associated with DPP II release were detectable by electron microscopy but not by light microscopy. Acrosin, which is less soluble than DPP II, is released at a later time during the acrosome reaction. Both DPP II and acrosin appear to be partially inhibited following their release from sperm. A complete understanding of the sequential release and extracellular activities of the acrosomal enzymes will be necessary to fully define their functions in fertilization.

L1 ANSWER 12 OF 25

AN 88:456950 BIOSIS
DN BA86:98669
TI FLUOROMETRIC EVALUATION OF CRYOPRESERVED BOVINE SPERMATOZOA EXTENDED IN EGG YOLK AND MILK.
AU GARNER D L; JOHNSON L A; ALLEN C H
CS UNIV. NEV.-RENO, RENO, NEV. 89557.
SO THERIOGENOLOGY 30 (2). 1988. 369-378. CODEN: THGNBO ISSN: 0093-691X
LA English
AB A comparison of fluorogenically quantifiable parameters of cryopreserved, bovine spermatozoa that had been processed in homogenized milk and egg yolk citrate-based extenders was made using flow cytometry. Semen from four bulls was processed in egg yolk-citrate or homogenized milk extenders, packaged in straws and frozen at -196.degree.C. Samples were thawed at ***37*** . ***degree*** .C, subdivided into three portions and stained after 0, 1.5 and 3 h of incubation at ***37*** . ***degree*** .C. ***Spermatozoa*** were ***stained*** using a combination of carboxyfluorescein diacetate (CFDA) and propidium iodide (PI) and analyzed by dual parameter flow cytometry. The sperm cells were quantified fluorometrically at each time interval for both green and red fluorescence. The proportion of spermatozoa retaining the fluorescent CFDA derivative was larger at each time interval for samples in egg yolk citrate than those in milk. Differences in the retention of spermatozoal viability were detected between identical samples of bovine spermatozoa extended in milk or egg yolk based media.

L1 ANSWER 13 OF 25

AN 88:305642 BIOSIS
DN BA86:22680
TI IDENTIFICATION OF ACROSOME-REACTED GOAT SPERMATOZOA BY A TRYPAN BLUE-GIEMSA METHOD.
AU KUSUNOKI H; SAKAUE M; HARAYAMA H; KATO S; KANDA S
CS THE GRAD. SCH. SCI. TECHNOL., KOBE UNIV., KOBE-SHI 657, JPN.
SO JPN J ZOOTECH SCI 59 (3). 1988. 235-240. CODEN: NICKA3 ISSN: 0369-4062
LA Japanese
AB The purpose of this study was to develop a new technique for identifying acrosome-reacted goat spermatozoa. Ejaculated spermatozoa were washed and incubated in an airtight glass tube at ***39*** .5. ***degree*** . C for 1, 2 and 3 hrs for inducing the acrosome reaction, and washed and frozen-thawed three times. Sperm samples were incubated with trypsan blue for 15 min, smeared on a slide, fixed in ORTH solution for 45 min, and finally stained in Giemsa solution (trypan blue-Giemsa method). ***Stained*** smears had ***spermatozoa*** with the following four ***staining*** patterns: a) unstained or light blue postacrosomal regions with reddish purple acrosomes (live sperm with normal acrosomes); b) unstained or light blue postacrosomal regions with unstained acrosomal regions or damaged acrosomes (live sperm without normal acrosomes); c) dark blue postacrosomal regions with dark reddish purple acrosomes (dead sperm with normal acrosomes); d) dark blue postacrosomal regions with dark blue acrosomal regions or damaged acrosomes (dead sperm without normal acrosomes). There was a significant positive partial correlation between the percentage of live sperm without normal acrosomes (category b) and that of zona-free hamster eggs penetrated by spermatozoa ($r = 0.4179$, $P < 0.01$, $n = 44$). Additionally, there was no difference between the results obtained by means of the present method and a triple-stain technique, while the staining procedure and solution preparation were much simpler in the former method than in the latter. These results indicate that the trypan blue-Giemsa method can be used to identify acrosome-reacted goat spermatozoa.

L1 ANSWER 14 OF 25

AN 87:468193 BIOSIS
DN BA84:113633
TI PENETRATION OF ZONE-FREE HAMSTER EGGS BY LIPOSOME-TREATED SPERM FROM THE BULL RAM STALLION AND BOAR.
AU GRAHAM J K; FOOTE R H; HOUGH S R
CS 204 MORRISON HALL, CORNELL UNIV., ITHACA, N.Y. 14853.
SO BIOL REPROD 37 (1). 1987. 181-188. CODEN: BIREBV ISSN: 0006-3363
LA English
AB Spermatozoa from each of four rams, four stallions, and three boars (six semen samples) were treated with dilauroylphosphatidylcholine (PC12) liposomes and compared with control bull sperm to induce the acrosome reaction (AR) and study possible penetration of the sperm into zona-free hamster eggs. Diluted sperm were incubated with several concentrations of PC12 for 7 min at ***39*** . ***degree*** . C prior to [redacted]semination of the hamster eggs[redacted] vitro.

The sperm from the bull were diluted to 106 cells/ml as previously studied. Sperm from the ram, stallion, and boar were diluted to 6 .times. 106 and 20 .times. 106 celkls/ml. After addition to the eggs, the sperm concentration was reduced by 75 percent. Inseminated eggs were incubated with sperm for ***3*** h at ***39*** . ***degree*** . C prior to being fixed, ***stained*** , and observed for ***sperm*** penetration. At an initial concentration of 6 .times. 106 cells/ml, bull sperm treated with 36.7 .mu.M PC12 achieved an egg penetration rate of 92%, whereas under nearly identical conditions stallion spermatozoa achieved only 54% egg penetration. Under similar conditions, ram spermatozoa failed to penetrate eggs, but when the initial sperm concentration was increased to 20 .times. 106 cells/ml, sperm incubated with 51.5 .mu.M PC12 achieved 52% egg penetration. Boar spermatozoa treated with PC12 at either sperm concentration failed to exhibit an AR or penetrate hamster eggs. In general, as PC12 concentration increased the percentage of sperm with an AR increased and sperm motility decreased. It is concluded that 1) PC12 lipsosomes are effective in inducing the AR in sperm from the bull, ram, and stallion, but under conditions tested are ineffective with boar sperm; 2) these acrosome-reacted spermatozoa will penetrate zona-free hamster eggs and so may provide insight into the fertilizing ability of sperm from individual males of several species.

L1 ANSWER 15 OF 25

AN 87:235390 BIOSIS
DN BA83:123560
TI EFFECT OF SECRETORY PARTICLES IN BOVINE SEMINAL VESICLE SECRETION ON SPERM MOTILITY AND ACROSOME REACTION.
AU AGRAWAL Y; VANHA-PERTTULA T
CS DEP. ANATOMY, UNIV. KUOPIO, P.O. BOX 6, 70211 KUOPIO, FINLAND.
SO J REPROD FERTIL 79 (2). 1987. 409-420. CODEN: JRPFA4 ISSN: 0022-4251
LA English
AB Particles found in bovine seminal vesicle secretion were enriched by centrifugation. They varied in size and morphology and contained Mg2+, Ca2+-activated ATPase, aminopeptidase A, alanyl aminopeptidase, .gamma.-glutamyl transpeptidase and dipeptidyl peptidase IV activities. Hyperactivation of sperm motility and the acrosome reaction were induced by these particles in epididymal spermatozoa suspended in a modified Ringer medium. The hyperactivation, analysed with a microscopic slide test, started within minutes of exposure to membrane particles and continued for 3-4 h, during which time ***spermatozoa*** underwent the acrosome reaction. Acrosome ***staining*** , phase-contrast microscopy and transmission electron microscopy revealed that the acrosome reaction started within 60 min at ***37*** . ***degree*** .C and affected up to 80% of spermatozoa in 4 h. These membrane particles differed from those reported previously in other species in enzyme composition, function and organ of origin.

L1 ANSWER 16 OF 25

AN 87:192609 BIOSIS
DN BA83:100733
TI DILAUROYLPHOSPHATIDYLCHOLINE LIPOSOME EFFECTS ON THE ACROSOME

REACTION AND IN-VITRO PENETRATION OF ZONA-FREE HAMSTER EGGS BY BULL SPERM II. A FERTILITY ASSAY FOR FROZEN-THAWED SEMEN.

AU GRAHAM J K; FOOTE R H

CS 204 MORRISON HALL, CORNELL UNIV., ITHACA, N.Y. 14853.

SO GAMETE RES 16 (2). 1987. 147-158. CODEN: GAMRDC ISSN: 0148-7280

LA English

AB Frozen-thawed sperm from five bulls with fertility rates ranging from 48% to 77% were treated with seven concentrations of dilauroylphosphatidylcholine (PC12) liposomes to induce an acrosome reaction (AR) that enabled sperm to penetrate eggs. Treated sperm were incubated with liposomes for 7 min prior to insemination of zona-free hamster eggs in vitro. Sperm and eggs were incubated ***3*** hr at ***39*** . ***degree*** . C prior to fixation, ***staining*** , and examination for ***sperm*** penetration and nuclear decondensation. The percentage of motile sperm immediately after thawing as well as after treatment with liposomes had a low correlation with sire fertility (r = 0.39 and .ltoreq. 63, respectively). The percentage of sperm exhibiting an AR was more highly correlated with fertility (r .ltoreq. -.85). Similar correlations were found between fertility and the penetration rates of zona-free hamster eggs or the total number of penetrating sperm. When data for two high and for two lower fertility bulls were each grouped to increase information per data point the correlation between the PC12 concentration giving the maximum proportion of eggs penetrated and fertility was r = 0.92 (P < 0.05). The correlation between the PC12 concentration producing the most total sperm penetrating the eggs and fertility r = 0.97 (P < 0.05). It was concluded that PC12 liposomes induced an AR in bull sperm frozen-thawed in egg yolk extender. Frozen-thawed sperm from low fertility bulls require less PC12 to induce the AR and to penetrate zona-free hamster eggs than do sperm from higher fertility bulls. These differences in lipid requirements may help to provide a quick, direct laboratory assay method to estimate the fertility of frozen bull semen.

L1 ANSWER 17 OF 25

AN 87:192608 BIOSIS

DN BA83:100732

TI DILAUROYLPHOSPHATIDYLCHOLINE LIPOSOME EFFECTS ON THE ACROSOME REACTION AND IN-VITRO PENETRATION OF ZONA-FREE HAMSTER EGGS BY BULL SPERM I. A FERTILITY ASSAY FOR FRESH SEMEN.

AU GRAHAM J K; FOOTE R H

CS 204 MORRISON HALL, CORNELL UNIV., ITHACA, N.Y. 14853.

SO GAMETE RES 16 (2). 1987. 133-146. CODEN: GAMRDC ISSN: 0148-7280

LA English

AB Fresh sperm from five bulls having nonreturn rates ranging from 48% to 77% were treated with 15.7, 21.0, 26.2, 31.5, 36.7 and 42.0 .mu.M dilauroylphosphatidylcholine (PC12) to induce the sperm acrosome reaction (AR). Treated sperm were incubated 3 hr with zona-free hamster eggs at ***39*** . ***degree*** . C prior to fixation. The eggs were then ***stained*** and examined for ***sperm*** penetration. Differences in the percentages of motile sperm and of sperm exhibiting an AR among bulls were small when compared on a within-liposome-concentration basis. Increasing the PC12 concentration from 15.7 .mu.M to 42.0 .mu.M increased the percentage

of sperm exhibiting an AR for all bulls. At the lowest lipid concentration (15.7 .mu.M), the percentage of eggs penetrated by sperm from the five bulls was 6% to 36%, with 0% in controls. When sperm were incubated with increasing lipid concentrations, the egg penetration rate increased to over 80%, and the total number of sperm increased to over 100 per 36 eggs in each treatment for every bull. These penetration rates decreased at the highest lipid concentration. A correlation between the PC12 concentration maximizing egg penetration and the nonreturn rate of -.63 was found. The correlation between the PC12 concentration maximizing the total number of penetrated sperm per treatment and the bull nonreturn rate was -.96. It was concluded that PC12 liposomes induce the AR in bull spermatozoa, which enables them to penetrate zona-free hamster eggs. High fertility bulls required less lipid to induce the AR than did lower fertility bulls. Consequently, this assay of fresh semen could provide a laboratory method to estimate the fertility of a bull.

L1 ANSWER 18 OF 25

AN 87:185104 BIOSIS
DN BA83:93228
TI SPERMATIC AGGLUTINATION PRODUCED BY SERUM FROM BULLS WITH TESTICULAR ABNORMALITIES.
AU FREGONESI J A; DA FONSECA J; RODRIGUES L H; PINHEIRO L E L; LEITE F G; ESPER C R
CS DEP. CLINICA E CIRURGIA VETERINARIA DA FAC. DE CIENCIAS AGRARIAS E VETERINARIAS "CAMPUS" DE JABOTICABAL, RODOVIA CARLOS TONANNI, KM 5-14.870 JABOTICABAL, SP.
SO ARS VET 2 (1). 1986. 35-39. CODEN: ARSVE6
LA Portuguese
AB Serum from 4 bulls with the testicular or epididymal abnormalities, diluted 1:1 in semen from normal bulls showing similar sperm characteristics, were incubate during 1 hour at ***37*** . ***degree*** . C. Under phase contrast microscopy, or using stained smears, a consistent head to head model of agglutination was observed. The frequency of sperm pathology was not a significant as the incidence of agglutination refers. However, semen with low cell concentration reacted more intensively than highly concentrated semen ($P < 0.05$). Both methods for ***sperm*** analysis (phase contrast and ***stained*** smear) did not differ as a process to identify this type of abnormality.

L1 ANSWER 19 OF 25

AN 86:197092 BIOSIS
DN BA81:88392
TI FERTILITY RESULTS WITH DEEP FROZEN BULL SEMEN AFTER DIFFERENT LENGTHS OF INCUBATION IN THE THAWING WATER.
AU MORKHOLM E; FILSETH O
CS NRF-DER NORWEGISCHE ROTVICH ZUCHTVERBAND, 2300 HAMAR, NORWEGEN.
SO ZUCHTHYGIENE (BERL) 20 (5). 1985 (RECD. 1986). 229-233. CODEN: ZUCYAN ISSN: 0044-5371
LA German
AB The common opinion that deep frozen bull semen must be used for insemination immediately after thawing creates practical problems in areas with bad communications. Two field trials using different

intervals between thawing and insemination were performed to see how fertility results were influenced by prolonged intervals. The following codes refer to how long time the semen dose was left before insemination in the thawing bath at a temperature of ***35*** . ***degree*** .- ***38*** . ***degree*** . C: Code 1: 15 sec-30 min; Code 2: 30 min-60 min; Code 3: 60 min-90 min; Code 4: 90 min-120 min. Even if an eosin-nigrosin differential staining revealed a successive increase of ***stained*** (dead) ***spermatozoa*** up to one and a half hour of incubation, the 60 days non-return results did not decrease as a result of this incubation.

L1 ANSWER 20 OF 25

AN 86:127760 BIOSIS
DN BA81:38176
TI THE EFFECT OF BUFFER OSMOLALITY ON THE SURVIVAL OF CAT FELIS-CATUS SPERMATOZOA AT 5 CELSIUS.
AU GLOVER T E; WATSON P F
CS DEP. PHYSIOL. ROYAL VET. COLL. ROYAL COLL. ST., LONDON NW1 0TU, UK.
SO THERIOGENOLOGY 24 (4). 1985. 449-456. CODEN: THGNBO ISSN: 0093-691X
LA English
AB Cat semen was diluted at ***37*** . ***degree*** . C in Tes-Tris buffer (Test), pH 7.5, at osmolalities ranging from 195 to 390 mOsm/kg, cooled to 5.degree. C over 90 minutes and stored for 24 hours at that temperature. Motility and percentage of ***spermatozoa*** ***staining*** with a supravital ***stain*** were estimated before cooling, after cooling and after storage for 24 hours. The osmolality of undiluted pooled ejaculates from five animals was measured, and also that of different diluents (citrate with phosphate buffer, lactose and TesT-egg yolk) used for cat semen. The osmolality measurements of cat semen suggested an osmolality of less than 320 mOsm/kg at ejaculation, increasing with time after ejaculation. Varying the egg yolk concentrations (2% to 20%) did not affect the osmolality of TesT diluent. Diluent osmolalities of less than 292 mOsm/kg were found to reduce sperm motility significantly ($P < 0.001$) although there was no significant increase in the percentage of cells staining with a supravital stain, while those greater than 325 mOsm/kg increased the variation of response among animals. Cooling and storage significantly reduced motility ($P < 0.01$ to $P < 0.001$) and increased the number of stained cells ($P < 0.001$). There were significant differences between ejaculates ($P < 0.01$) and significant interactions between osmolality and cooling/storage ($P < 0.05$ to $P < 0.001$). The best overall results were seen with a TesT diluent of 292 to 325 mOsm/kg which supported good motility for at least 24 hours.

L1 ANSWER 21 OF 25

AN 83:315887 BIOSIS
DN BA76:73379
TI METHANOL ACETIC ANHYDRIDE AN EFFICIENT BLOCKING AGENT FOR ELECTRON MICROSCOPE CYTOCHEMISTRY ITS APPLICATION TO MOUSE TESTIS AND OTHER TISSUES.
AU TANDLER C J; SOLARI A J
CS INSTITUTO DE BIOLOGIA CELLULAR AND CIR, FACULTAD DE MEDICINA, 1121, BUENOS AIRES, ARGENTINA.

SO HISTOCHEMISTRY 76 (3). 1982 (RECD. 1983). 351-362. CODEN: HCMYAL ISSN: 0301-5564

LA English

AB A mixture of absolute methanol and acetic anhydride (MA) (5:1 vol/vol; 24 h at 25.degree. C) which is known to methylate and acetylate, respectively, free carboxyl and amino groups in proteins, was tested for its effectiveness as a blocking agent in glutaraldehyde-fixed mouse testis and skeletal muscle. In young ***spermatids*** , the ***staining*** of the acrosome with either uranyl acetate (UA) or ethanolic phosphotungustic acid (PTA) was completely prevented by prior treatment with MA. Esterification of carboxyl groups with 0.1 N HCl in methanol (24 h at ***30*** . ***degree*** . C) eliminated the UA staining without affecting that due to PTA. Apparently, COOH groups are responsible for UA binding and acetylation (of amino groups) prevented PTA binding. MA also abolishes the strong affinity of PTA to the lateral elements of the synaptonemal complex in meiotic chromosomes, the axoneme and fibrous sheath of the spermatid tail and the Z band in skeletal muscle. Reactivity was diminished in nucleoli and remained unaffected in chromatin, the outer coarse fibers of the flagellum and collagen fibrils. Different functional groups may participate in PTA staining. The ultrastructure was well preserved in all cases.

L1 ANSWER 22 OF 25

AN 83:192602 BIOSIS

DN BA75:42602

TI INFLUENCE OF PSEUDOMONAS-AERUGINOSA STAPHYLOCOCCUS-AUREUS AND ESCHERICHIA-COLI TYPES ON METABOLIC PROCESSES AND VITALITY OF SPERMATOZOA.

AU BORYCZKO Z

CS AKADEMIA ROLNICZA W SZCEZECINIE, INSTYTUT HODOWLI I TECHNOLOGII PRODUKCJI ZWIERZECEJ, 71-460 SZCEZECIN, UL. DR. JUDYMA NR. 6, POLEN.

SO ZUCHTHYGIENE (BERL) 17 (1). 1982. 13-18. CODEN: ZUCYAN ISSN: 0044-5371

LA German

AB Bull semen was incubated with P. aeruginosa, S. aureus and E. coli cultures in warburg's apparatus at ***37*** . ***degree*** . C for 60 min. The effect of these microorganisms at a concentration of 3 or 30 .times. 106/ml diluted semen on respiration of spermatozoa, glutamic oxalacetic transaminase (GOT) activity in seminal plasma and live-dead ***staining*** of ***spermatozoa*** was examined. A considerable reduction of the percentage of live spermatozoa was observed following incubation of semen samples with 30 .times. 106 of these microorganisms. In comparison with the control, the differences amounted to 6.9% for Pseudomonas ($P < 0.05$), 9.7% for Staphylococcus ($P < 0.01$) and 6.5% for E. coli ($P < 0.01$). The tested microorganisms reduced the O2 uptake of spermatozoa (respiration of the microorganisms was taken into account). A decrease in the O2 uptake in semen samples with 30 .times. 106 Staphylococcus was observed after 15 min of incubation. Following 60 min incubation, O2 consumption was considerably lower in semen samples with 30 .times. 106 of all examined microorganisms compared controls ($P < 0.01$). GOT activity in the semen samples increased after incubation with 30 .times. 106 of P. aeruginosa and S. aureus ($P < 0.01$).

L1 ANSWER 23 OF 25

AN 83:164599 BIOSIS
DN BA75:14599
TI ATP ADP AND AMP CONTENT OF SPERMATOZOA OF DOMESTIC ANIMALS AND MAN AND OF BULL SPERMATOZOA DURING DEEP FREEZE PRESERVATION.
AU KAEHN W; PFETSCH J; LEIDL W
CS GYNAEKOL., AMBULATORISCHE TIERKLINIK, UNIV. MUENCHEN, KOENIGINSTR. 12, 8000 MUENCHEN 22.
SO ZUCHTHYGIENE (BERL) 17 (2). 1982. 49-55. CODEN: ZUCYAN ISSN: 0044-5371
LA German
AB The content of ATP, ADP and AMP in sperm of men, bulls, stallions, rams, goats, rabbits, boars, dogs and cocks was investigated by means of the bioluminescent measurement. The content of ATP was higher than ADP or AMP in all species except the cock. The adenylate energy charge was between 0.6 and 0.75. The ATP level was correlated with the usual criteria, motility estimation and supravital ***staining*** for ***sperm*** stored at + ***38*** . ***degree*** . C for 18 h. During the deepfreezing of bull semen, the unfractioned addition of glycerol or cooling were more clear determinated by a fall in ATP level than a reduction in motility. The 3 adenosine nucleotides, ATP, ADP and AMP reacted differnetly during the freezing procedure; the ATP level fell, the ADP level remained nearly constant, and the AMP level increased in the course of deepfreezing and fell after thawing.

L1 ANSWER 24 OF 25

AN 80:254854 BIOSIS
DN BA70:47350
TI EFFECT OF CATALASE IN DILUENT ON SURVIVAL AND ACROSOME SYSTEM OF BOAR SPERMATOZOA STORED AT 4 CELSIUS.
AU KATO S; IKEGAMI J; SAIDA J
CS COLL. AGRIC., KYOTO UNIV., KYOTO 606, JPN.
SO JPN J ANIM REPROD 25 (3). 1979. 120-125. CODEN: KHZADH ISSN: 0385-9932
LA Japanese
AB The effects of catalase with and without reduced glutathione (GSH), ethylenediaminetetraacetate (EDTA) or sodium laurylsulfate (SLS) in diluent on the survival and acrosome system of boar spermatozoa stored at 4.degree. C were investigated. Semen was collected from 4 Landrace boars by the manual method and only sperm-rich fraction was used. The basic diluent for all experiments contained 20 ml egg yolk, 1.075 g Tris, 0.533 g citric acid, 2.667 g glucose, 100,000 IU potassium penicillin G and 0.1 g dihydrostreptomycin sulfate per 100 ml of diluent. The sperm samples were cooled to room temperature (18 .apprx. 23.degree. C) at a constant rate of 0. ***33*** . ***degree*** . C/min, centrifuged for 10 .apprx. 15 min at 600 g and resuspended in the basic diluent to give a concentration of 10 .times. 108 cells per ml. The sperm suspension was diluted 1:4 (suspension: diluent) with the diluent containing different levels of catalase and/or other agents and gradually cooled to 4.degree. C over 4 h. After 5, 10 and 15 days of storage at 4.degree. C, sperm motility was assessed microscopically at 15 min intervals during incubation at ***37*** ***degree*** . C for 60 .appr 90 min.

Acrosome morphology of ***spermatozoa*** was assessed after ***staining*** in a 7.5% (V/V) buffered solution of Giemsa stain. In 3 experiments, the effects of catalase (300 sigma units/ml) in diluent containing different levels of GSH (0, 5, 10 and 20 mM), EDTA (0, 2.5, 5 and 10 mM) or SLS (0, 0.02, 0.04, 0.08 and 0.16% W/V) on sperm survival were examined. The addition of catalase improved sperm survival. GSH and SLS prolonged sperm life when they were used singly, but the beneficial effect of these agents was reduced more rapidly than that of catalase alone. Both GSH and SLS had no beneficial effect when they were combined with catalase. EDTA was not beneficial to sperm survival regardless of the presence or absence of catalase.

L1 ANSWER 25 OF 25

AN 78:162040 BIOSIS
DN BA65:49040
TI THE EFFECT OF TEMPERATURE ON SPERM MOTILITY AND VIABILITY.
AU APPELL R A; EVANS P R
CS SECT. UROL., YALE-NEW HAVEN HOSP., 789 HOWARD AVE., NEW HAVEN, CONN. 06504, USA.
SO FERTIL STERIL 28 (12). 1977 (RECD 1978) 1329-1332. CODEN: FESTAS ISSN: 0015-0282
LA English
AB Semen specimens from fertile prevasectomy patients maintained at 4. ***degree*** ., 20. ***degree*** . and ***37*** . ***degree*** . C were evaluated at 3, 6, 12 and 18 h after collection. ***Sperm*** viability, assessed by eosin-nigrosin ***stain*** , and motility decreased with time at 20. ***degree*** . and ***37*** . ***degree*** . C, but at a significantly higher rate at ***37*** . ***degree*** . C (where the motility was halved by 12 h). The slope of the decrease in viability closely paralleled that of the motility except at 4.degree. C, where motility was nearly absent at 6 h but viability was retained through 18 h. Bacterial counts rose markedly and the pH fell at ***37*** . ***degree*** . C, which may explain the decrease in motility and viability. Semen should be kept at room temperature (20.degree. C) and not at ***37*** . ***degree*** . C if there is to be any delay in its analysis, or a falsely lowered motility will result.

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      (FILE 'HOME' ENTERED AT 13:32:18 ON 30 AUG 91)

      FILE 'BIOSIS' ENTERED AT 13:32:30 ON 30 AUG 91
L1             25 (STAIN?(5A)SPERM?) AND (3#(3A)DEGREE#)

      FILE 'CA' ENTERED AT 13:54:13 ON 30 AUG 91
L2              0 (STAIN?(5A)SPERM?) AND DEGREE#
L3              0 (STAIN?(5A)SPERM?) AND TEMPERAT?

=>
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L2        2407 JOHNSON, L?/AU

=> l2 and (sort?(10a)sperm?)

          7268 SORT?
         49935 SPERM?
            32 SORT?(10A)SPERM?
L3          14 L2 AND (SORT?(10A)SPERM?)

=> d 1-14


L3  ANSWER 1 OF 14

AN  90:230452  BIOSIS
DN  BR38:108590
TI  A FLOW CYTOMETRIC-  ***SORTING***  METHOD FOR SEXING MAMMALIAN
    ***SPERM***  VALIDATED BY DNA ANALYSIS AND LIVE BIRTHS.
AU    ***JOHNSON L A***
CS  U.S. DEP. AGRIC., ARS, REPRODUCTION LAB., BELTSVILLE, MD. 20705.
SO  XIVTH INTERNATIONAL MEETING OF THE SOCIETY FOR ANALYTICAL CYTOLOGY,
    ASHEVILLE, NORTH CAROLINA, USA, MARCH 18-23, 1990. CYTOMETRY 0
    (SUPPL. 4). 1990.  42. CODEN: CYTODQ ISSN: 0196-4763
DT  Conference
LA  English

L3  ANSWER 2 OF 14

AN  90:17896  BIOSIS
DN  BR38:7196
TI  ALTERED SEX RATIOS IN OFFSPRING AFTER SURGICAL INSEMINATION OF FLOW-
    ***SORTED***  POPULATIONS OF X AND Y CHROMOSOME-BEARING
    ***SPERM***  .
AU    ***JOHNSON L A***  ; FLOOK J P; HAWK H W
CS  REPRODUCTION LAB., AGRIC. RES. SERV., U.S. DEP. AGRIC., BELTSVILLE,
    MD. 20705.
SO  TWENTY-SECOND ANNUAL MEETING OF THE SOCIETY FOR THE STUDY OF
    REPRODUCTION, COLUMBIA, MISSOURI, USA, AUGUST 6-9, 1989. BIOL REPROD
    40 (SUPPL. 1). 1989.  162. CODEN: BIREBV ISSN: 0006-3363
DT  Conference
LA  English

L3  ANSWER 3 OF 14

AN  90:272  BIOSIS
DN  BA89:272
TI  SEX PRESELECTION IN RABBITS LIVE BIRTHS FROM X AND Y  ***SPERM***
    SEPARATED BY DNA AND CELL  ***SORTING***  .
AU    ***JOHNSON L A***  ; FLOOK J P; HAWK H W
CS  REPROD. LAB., BELTSVILLE AGRIC. RES. CENT., AGRIC. RES. SERV., U.S.
    DEP. AGRIC., BELTSVILLE, MD. 20705, USA.
SO  BIOL REPROD 41 (2). 1989.  199-203. CODEN: BIREBV ISSN: 0006-3363
LA  English

L3  ANSWER 4 OF 14
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8/89
10/89

QP251.B5

AN 87:414485 BIOSIS
DN BR33:84163
TI FLOW ***SORTING*** OF SONICATED X AND Y CHROMOSOME-BEARING MAMMALIAN ***SPERM*** BASED ON RELATIVE DNA CONTENT.
AU ***JOHNSON L A*** ; FLOOK J P; CLARKE R N; LOOK M V
CS U.S. DEP. AGRIC., ARS, REPRODUCTION LAB., BELTSVILLE, MD., USA.
SO XIITH INTERNATIONAL MEETING OF THE SOCIETY FOR ANALYTICAL CYTOLOGY, CAMBRIDGE, ENGLAND, UK, AUGUST 9-15, 1987. CYTOMETRY 0 (SUPPL. 1). 1987. 88. CODEN: CYTODQ ISSN: 0196-4763
DT Conference
LA English

L3 ANSWER 9 OF 14

AN 87:385255 BIOSIS
DN BA84:71752
TI FLOW CYTOMETRY OF X AND Y CHROMOSOME-BEARING SPERM FOR DNA USING AN IMPROVED PREPARATION METHOD AND STAINING WITH HOECHST 33342.
AU ***JOHNSON L A*** ; FLOOK J P; LOOK M V
CS USDA-ARS, ANIMAL SCI. INST., REPRODUCTION LAB., BUILDING 200, BARC-EAST, BELTSVILLE, MD 20705.
SO GAMETE RES 17 (3). 1987. 203-212. CODEN: GAMRDC ISSN: 0148-7280
LA English

L3 ANSWER 10 OF 14

AN 87:308103 BIOSIS
DN BR33:29776
TI FLOW CYTOMETRY ANALYSIS AND ***SORTING*** OF X AND Y CHROMOSOME-BEARING ***SPERMATOZOA*** .
AU ***JOHNSON L A***
CS U.S. DEP. AGRIC., REPRODUCTION LAB., ANIMAL SCI. INST., BELTSVILLE AGRIC. RES. CENT., AGRIC. RES. SERV., BELTSVILLE, MD. 20705, USA.
SO AUGUSTINE, P. C., H. D. DANFORTH AND M. R. BAKST (ED.). BELTSVILLE SYMPOSIA IN AGRICULTURAL RESEARCH, 10. BIOTECHNOLOGY FOR SOLVING AGRICULTURAL PROBLEMS; BELTSVILLE, MARYLAND, USA, MAY 5-9, 1985. XIII+416P. MARTINUS NIJHOFF PUBLISHERS: DORDRECHT, NETHERLANDS (DIST. FOR USA AND CANADA BY KLUWER ACADEMIC PUBLISHERS: HINGHAM, MASSACHUSETTS, USA; FOR THE UK AND IRELAND BY KLUWER ACADEMIC PUBLISHERS, MTP PRESS LTD.: LANCASTER, ENGLAND, UK). ILLUS. 0 (0). 1986 (RECD. 1987). 121-134. CODEN: BSARDN ISBN: 90-247-3311-1 ISSN: 0160-3612
LA English

L3 ANSWER 11 OF 14

AN 87:167235 BIOSIS
DN BA83:85676
TI FLOW ***SORTING*** OF X AND Y CHROMOSOME-BEARING ***SPERMATOZOA*** INTO TWO POPULATIONS.
AU ***JOHNSON L A*** ; FLOOK J P; LOOK M V; PINKEL D
CS USDA-ARS, ANIM. SCI. INST., REPRODUCTION LAB., BUILD. 200, BARC-EAST, BELTSVILLE, MD. 20705.
SO GAMETE RES 16 (1). 1987. 1-10. CODEN: GAMRDC ISSN: 0148-7280
LA English

AN 89:69035 BIOSIS
DN BA87:33433
TI FLOW ***SORTING*** OF X AND Y CHROMOSOME-BEARING MAMMALIAN ***SPERM*** ACTIVATION AND PRONUCLEAR DEVELOPMENT OF ***SORTED*** BULL BOAR AND RAM ***SPERM*** MICROINJECTED INTO HAMSTER OOCYTES.
AU ***JOHNSON L A*** ; CLARKE R N
CS USDA-ARS REPRODUCTION LAB., BELTSVILLE AGRIC. RES. CENT., BUILD. 200, BELTSVILLE, MD. 20705.
SO GAMETE RES 21 (4). 1988. 335-344. CODEN: GAMRDC ISSN: 0148-7280
LA English

L3 ANSWER 5 OF 14

AN 88:475562 BIOSIS
DN BR35:105452
TI FLOW ***SORTING*** OF INTACT X AND Y CHROMOSOME-BEARING MAMMALIAN ***SPERMATOZOA*** .
AU ***JOHNSON L A***
CS U.S. DEP. AGRIC., ARS, REPRODUCTION LAB., BELTSVILLE, MD.
SO XII INTERNATIONAL MEETING OF THE SOCIETY FOR ANALYTICAL CYTOLOGY, BRECKENRIDGE, COLORADO, USA, SEPTEMBER 4-9, 1988. CYTOMETRY 0 (SUPPL. 2). 1988. 66. CODEN: CYTODQ ISSN: 0196-4763
DT Conference
LA English

L3 ANSWER 6 OF 14

AN 88:27908 BIOSIS
DN BA85:15633
TI INCIDENCE OF CHROMOSOME ABERRATIONS IN MAMMALIAN ***SPERM*** STAINED WITH HOECHST 33342 AND UV-LASER IRRADIATED DURING FLOWING ***SORTING*** .
AU LIBBUS B L; PERREAULT S D; ***JOHNSON L A*** ; PINKEL D
CS REPROD. LAB., AGRIC. RES. SERV., U.S. DEP. AGRIC., BELTSVILLE, MD 20705, USA.
SO MUTAT RES 182 (5). 1987. 265-274. CODEN: MUREAV ISSN: 0027-5107
LA English

L3 ANSWER 7 OF 14

AN 88:17214 BIOSIS
DN BR34:5724
TI SEPARATION OF X AND Y CHROMOSOME-BEARING MAMMALIAN ***SPERM*** BY DNA CONTENT USING FLOW CYTOMETRIC ANALYSIS AND ***SORTING*** .
AU ***JOHNSON L A***
CS U.S. DEP. AGRIC., AGRIC. RES. SERVICE, REPRODUCTION LAB., BELTSVILLE, MD.
SO SYMPOSIUM ON FETAL-MATERNAL RELATIONSHIPS HELD AT THE TWENTIETH ANNUAL MEETING OF THE SOCIETY FOR THE STUDY OF REPRODUCTION, URBANA, ILLINOIS, USA, JULY 20-23, 1987. BIOL REPROD 36 (SUPPL. 1). 1987. 80. CODEN: BIREBV ISSN: 0006-3363
DT Conference
LA English

L3 ANSWER 8 OF 14

L3 ANSWER 12 OF 14

AN 87:35401 BIOSIS
DN BR32:15489
TI FLOW ***SORTING*** OF X AND Y CHROMOSOME BEARING ***SPERM***
INTO SEPARATE POPULATIONS OF THE BASIS OF DNA CONTENT.
AU ***JOHNSON L A***
CS US DEPT. OF AGRICULTURE, AGRIGULTURAL RESEARCH SERVICE, REPRODUCTION
LABORATORY, BELTSVILLE, MD. USA.
SO SECOND EUROPEAN CONGRESS ON CELL BIOLOGY, BUDAPEST, HUNGARY, JULY
6-11, 1986. ACTA BIOL HUNG 37 (SUPPL.). 1986. 93. CODEN: ABHUE6
ISSN: 0236-5383
DT Conference
LA English

L3 ANSWER 13 OF 14

AN 86:279451 BIOSIS
DN BA82:23314
TI MODIFICATION OF A LASER-BASED FLOW CYTOMETER FOR HIGH-RESOLUTION DNA
ANALYSIS OF MAMMALIAN SPERMATOZOA.
AU ***JOHNSON L A*** ; PINKEL D
CS USDA-ARS, ANIMAL SCI. INST., REPRODUCTION LAB., BUILD. 200, BARC-EAST
BELTSVILLE, MD. 20705.
SO CYTOMETRY 7 (3). 1986. 268-273. CODEN: CYTODQ ISSN: 0196-4763
LA English

L3 ANSWER 14 OF 14

AN 85:136742 BIOSIS
DN BR29:26738
TI FLOW ***SORTING*** OF X AND Y CHROMOSOME-BEARING CHINCHILLA
SPERMATOZOA INTO 2 POPULATIONS.
AU ***JOHNSON L A*** ; FLOOK J; LOOK M; PINKEL D
CS USDA, AGRIC. RES. SERVICE, REPRODUCTION LAB., BELTSVILLE, MD 20705.
SO 3RD INTERNATIONAL CONGRESS OF ANDROLOGY, BOSTON, MASS., USA, APR.
27-MAY 2, 1985. J ANDROL 6 (2 SUPPL.). 1985. 128-P. CODEN: JOAND3
ISSN: 0196-3635
DT Conference
LA English

L1 ANSWER 1 OF 39

TI PREDETERMINATION OF SEX IN IN MAMMALS NEW PROSPECTS.
AN 91:158755 BIOSIS

L1 ANSWER 2 OF 39

TI FLOW CYTOMETRY AND ***SORTING*** OF DIPLOID ELONGATED ***SPERMATIDS*** AND OTHER TESTICULAR CELL TYPES IN THE MOUSE.
AN 91:105510 BIOSIS

L1 ANSWER 3 OF 39

TI FLOW CYTOMETRIC ***SORTING*** OF VIABLE PORCINE X-CHROMOSOME AND Y-CHROMOSOME-BEARING ***SPERM*** VALIDATED BY BIRTH OF OFFSPRING AND DNA ANALYSIS.
AN 91:43791 BIOSIS

L1 ANSWER 4 OF 39

TI ASSESSMENT BY FLUORESCENCE-ACTIVATED CELL ***SORTING*** OF WHETHER ***SPERM*** -ASSOCIATED IMMUNOGLOBULIN IGG AND IGA OCCUR ON THE SAME SPERM POPULATION.
AN 90:426411 BIOSIS

L1 ANSWER 5 OF 39

TI FLOW CYTOMETRY AND ***SORTING*** OF ***SPERM*** AND MALE GERM CELLS.
AN 90:388454 BIOSIS

L1 ANSWER 6 OF 39

TI ENRICHMENT OF BOVINE X AND Y-CHROMOSOME-BEARING ***SPERM*** WITH MONOCLONAL H-Y ANTIBODY FLUORESCENCE-ACTIVATED CELL ***SORTER*** .
AN 90:359912 BIOSIS

L1 ANSWER 7 OF 39

TI ROUND-HEADED SPERMATOZOA A MODEL TO STUDY THE ROLE OF THE ACROSOME IN EARLY EVENTS OF GAMETE INTERACTION.
AN 90:291546 BIOSIS

L1 ANSWER 8 OF 39

TI A FLOW CYTOMETRIC- ***SORTING*** METHOD FOR SEXING MAMMALIAN ***SPERM*** VALIDATED BY DNA ANALYSIS AND LIVE BIRTHS.
AN 90:230452 BIOSIS

L1 ANSWER 9 OF 39

TI COMPARATIVE ULTRASTRUCTURAL INVESTIGATION OF THE EUSPERMATOZOA AND PARASPERMATOZOA OF 13 PROTODRILUS SPECIES POLYCHAETA ANNELIDA AND ITS TAXONOMICAL AND PHYLOGENETICAL IMPLICATIONS.

AN 90:70755 BIOSIS

L1 ANSWER 10 OF 39

TI FLOW CYTOMETRIC ANALYSIS OF MOUSE SPERM USING MONOCLONAL ANTI-SPERM ANTIBODY OBF13.
AN 90:32824 BIOSIS

L1 ANSWER 11 OF 39

TI OFFSPRING FROM INSEMINATIONS WITH MAMMALIAN SPERM STAINED WITH HOECHST 33342 EITHER WITH OR WITHOUT FLOW CYTOMETRY.
AN 90:27405 BIOSIS

L1 ANSWER 12 OF 39

TI ALTERED SEX RATIOS IN OFFSPRING AFTER SURGICAL INSEMINATION OF FLOW-***SORTED*** POPULATIONS OF X AND Y CHROMOSOME-BEARING ***SPERM*** .
AN 90:17896 BIOSIS

L1 ANSWER 13 OF 39

TI SEX PRESELECTION IN RABBITS LIVE BIRTHS FROM X AND Y ***SPERM*** SEPARATED BY DNA AND CELL ***SORTING*** .
AN 90:272 BIOSIS

L1 ANSWER 14 OF 39

TI RADIATION-INDUCED DIPLOID SPERMATIDS IN MICE.
AN 89:340844 BIOSIS

L1 ANSWER 15 OF 39

TI DETECTION OF SPONTANEOUS AUTOIMMUNITY OF BULLS IN CELLULAR RESPONSES.
AN 89:335547 BIOSIS

L1 ANSWER 16 OF 39

TI FEMALE ACCESSORY GLANDS WITH THEIR PROBABLE ROLE IN A SYRPHID FLY ERISALIS-ARVORUM FABR.
AN 89:313440 BIOSIS

L1 ANSWER 17 OF 39

TI FLOW ***SORTING*** OF X AND Y CHROMOSOME-BEARING MAMMALIAN ***SPERM*** ACTIVATION AND PRONUCLEAR DEVELOPMENT OF ***SORTED*** BULL BOAR AND RAM ***SPERM*** MICROINJECTED INTO HAMSTER OOCYTES.
AN 89:69035 BIOSIS

L1 ANSWER 18 OF 39

TI FLOW ***SORTING*** OF INTACT X AND Y CHROMOSOME-BEARING MAMMALIAN ***SPERMATOZOA*** .
AN 88:475562 BIOSIS

L1 ANSWER 19 OF 39

TI INCIDENCE OF CHROMOSOME ABERRATIONS IN MAMMALIAN ***SPERM***
STAINED WITH HOECHST 33342 AND UV-LASER IRRADIATED DURING FLOWING
SORTING .
AN 88:27908 BIOSIS

L1 ANSWER 20 OF 39

TI SEPARATION OF X AND Y CHROMOSOME-BEARING MAMMALIAN ***SPERM*** BY
DNA CONTENT USING FLOW CYTOMETRIC ANALYSIS AND ***SORTING*** .
AN 88:17214 BIOSIS

L1 ANSWER 21 OF 39

TI WATER AVAILABILITY AFFECTS REPRODUCTION IN DEER MICE.
AN 88:10398 BIOSIS

L1 ANSWER 22 OF 39

TI FLOW ***SORTING*** OF SONICATED X AND Y CHROMOSOME-BEARING
MAMMALIAN ***SPERM*** BASED ON RELATIVE DNA CONTENT.
AN 87:414485 BIOSIS

L1 ANSWER 23 OF 39

TI FLOW CYTOMETRY OF X AND Y CHROMOSOME-BEARING SPERM FOR DNA USING AN
IMPROVED PREPARATION METHOD AND STAINING WITH HOECHST 33342.
AN 87:385255 BIOSIS

L1 ANSWER 24 OF 39

TI FLOW CYTOMETRY ANALYSIS AND ***SORTING*** OF X AND Y
CHROMOSOME-BEARING ***SPERMATOZOA*** .
AN 87:308103 BIOSIS

L1 ANSWER 25 OF 39

TI THE SPERM LENGTH AND THE INTERNAL REPRODUCTIVE ORGANS OF DROSOPHILA
WITH SPECIAL REFERENCES TO PHYLOGENETIC RELATIONSHIPS.
AN 87:210797 BIOSIS

L1 ANSWER 26 OF 39

TI FLOW ***SORTING*** OF X AND Y CHROMOSOME-BEARING
SPERMATOZOA INTO TWO POPULATIONS.
AN 87:167235 BIOSIS

L1 ANSWER 27 OF 39

TI FLOW ***SORTING*** OF X AND Y CHROMOSOME BEARING ***SPERM***
INTO SEPARATE POPULATIONS OF THE BASIS OF DNA CONTENT.
AN 87:35401 BIOSIS

L1 ANSWER 28 OF 39

TI STANDARDS FOR SPERMIOGRAMS AND SPERMOCYTOGRAMS IN THE AFRICAN REGION OF SENEGAL.
AN 87:8802 BIOSIS

L1 ANSWER 29 OF 39

TI MODIFICATION OF A LASER-BASED FLOW CYTOMETER FOR HIGH-RESOLUTION DNA ANALYSIS OF MAMMALIAN SPERMATOZOA.
AN 86:279451 BIOSIS

L1 ANSWER 30 OF 39

TI OBAINIA-PETTERI NEW-SPECIES RHIGONEMATIDAE NEMATODA FROM PACHYBOLUS-LAMINATUS DIPLOPODA IN IVORY-COAST DESCRIPTION AND STUDY OF THE SPERMIOGENESIS.
AN 86:162755 BIOSIS

L1 ANSWER 31 OF 39

TI ULTRASTRUCTURE AND FORMATION OF THE BURSA MOUTHPIECE OF PHILOCELIS-CELLATA PLATYHELMINTHES ACOELA.
AN 86:143279 BIOSIS

L1 ANSWER 32 OF 39

TI COPULATION BY HYPODERMIC INJECTION IN THE NUDIBRANCHS PALIO-ZOSTERAE AND PALIO-DUBIA GASTROPODA OPISTHOBRANCHIA.
AN 85:316058 BIOSIS

L1 ANSWER 33 OF 39

TI FLOW ***SORTING*** OF X AND Y CHROMOSOME-BEARING CHINCHILLA ***SPERMATOZOA*** INTO 2 POPULATIONS.
AN 85:136742 BIOSIS

L1 ANSWER 34 OF 39

TI FLOW MICRO FLUOROMETRIC ANALYSIS OF LIVING SPERMATOZOA STAINED WITH HOECHST 33342.
AN 83:318713 BIOSIS

L1 ANSWER 35 OF 39

TI STUDIES ON HERMAPHRODITISM OF THE OVOVIVIPAROUS PULMONATE EUPHAEDUSA-TAU 1. SHELL WHORLS AS A GROWTH PARAMETER.
AN 83:222982 BIOSIS

L1 ANSWER 36 OF 39

TI SEX PRE SELECTION IN MAMMALS? SEPARATION OF SPERM BEARING Y AND O CHROMOSOMES IN THE VOLE MICROTUS-OREGONI.
AN 83:214557 BIOSIS

L1 ANSWER 37 OF 39

TI DNA FLOW CYTOMETRY OF HUMAN EJACULATES IN THE INVESTIGATION OF MALE

INFERTILITY.
AN 83:187798 BIOSIS

L1 ANSWER 38 OF 39

TI DNA ANALYSIS AND SORTING OF VIABLE MOUSE TESTIS CELLS.
AN 82:146488 BIOSIS

L1 ANSWER 39 OF 39

TI SPERMATOGONIAL RESPONSIVENESS TO MAMMALIAN GONADOTROPINS IN SUBADULT RANA-NIGROMACULATA.
AN 78:176562 BIOSIS

=> d 1 2 3 5 8 11 12 13 17 18 19 20 22 23 24 26 27 29 33 34 bib ab

L1 ANSWER 1 OF 39

AN 91:158755 BIOSIS
DN BA91:84555
TI PREDETERMINATION OF SEX IN IN MAMMALS NEW PROSPECTS.
AU BLOTTNER S; SCHWERIN M; MATZEL J; NEHRING H; PITRA C
CS FORSCHUNGSSTELLE WIRBELTIERFORSCHUNG, AM TIERPARK 125, BERLIN 01136.
SO BIOL ZENTRALBL 109 (6). 1990. 425-445. CODEN: BIZNAT ISSN: 0006-3304
LA German
AB The manipulation of sex ratio of offspring in mammals is possible in two ways at present: 1) preconceptionally by separation or selective influence on andro- or gynosperm and 2) postconceptionally by diagnosis and selection of sex of embryos. The biological prerequisites cause only minimal qualitative and quantitative differences between cells of different gonosome constellation. These consist in the primary difference of this gonosome content involving different mass (X-chromosome > Y-chromosome) and quality of gonosomal DNA. Secondary differences may result from gonosomal coded gene products. The most important results of sex preselection of the last 10 years are represented. Attempts to separate living X and Y sperm use different ***sperm*** motility, density or surface charge. Now the flow-cytometric cell ***sorting*** is very useful for separation with high purity. New prospects also result from application of sexing to the fertilization in vitro including microinjection of sperm for which extremely small numbers of sperm are needed. This permits a strong fractionation by means of methods with high selectivity. Besides improved methods to detect gonosomes in sperm and embyros have been developed allowing more exact control of separation success. Karyotypic analysis after heterologous fertilization, Y-specific DNA-probes und flow cytometry belong to these methods. The sex ratio also can be influenced by factors acting during fertilization and early embryonic development. Therefore the evidence of successful sex predetermination is necessary on three levels: for enrichment of X or Y sperm, for the gonosome content of fertilized oocytes and for the sex ratio at birth.

L1 ANSWER 2 OF 39

AN 91:105510 BIOSIS

DN BR40:48330
TI FLOW CYTOMETRY AND ***SORTING*** OF DIPLOID ELONGATED ***SPERMATIDS*** AND OTHER TESTICULAR CELL TYPES IN THE MOUSE.
AU TATCHEN I; HACKER-KLOM U; GOEHDE W
CS INST. STRAHLENBIOL., UNIV. MUENSTER, MUENSTER, FRG.
SO FIRST CONFERENCE OF THE EUROPEAN SOCIETY FOR ANALYTICAL CELLULAR PATHOLOGY, SCHLOSS ELMAU, WEST GERMANY, NOVEMBER 12-17, 1989. ANAL CELL PATHOL 1 (5-6). 1989. 341. CODEN: ACPAER
DT Conference
LA English

L1 ANSWER 3 OF 39

AN 91:43791 BIOSIS
DN BR40:20771
TI FLOW CYTOMETRIC ***SORTING*** OF VIABLE PORCINE X-CHROMOSOME AND Y-CHROMOSOME-BEARING ***SPERM*** VALIDATED BY BIRTH OF OFFSPRING AND DNA ANALYSIS.
AU JOHNSON L A
CS U.S. DEP. AGRIC., ARS, REPRODUCTION LAB., BELTSVILLE, MD. 20705.
SO THIRTIETH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CELL BIOLOGY, SAN DIEGO, CALIFORNIA, USA, DECEMBER 9-13, 1990. J CELL BIOL 111 (5 PART 2). 1990. 111A. CODEN: JCLBA3 ISSN: 0021-9525
DT Conference
LA English

L1 ANSWER 5 OF 39

AN 90:388454 BIOSIS
DN BR39:59415
TI FLOW CYTOMETRY AND ***SORTING*** OF ***SPERM*** AND MALE GERM CELLS.
AU GLEDHILL B L; EVENSON D P; PINKEL D
CS BIOMED. SCI. DIV., LAWRENCE LIVERMORE NATL. LAB., LIVERMORE, CALIF. 94550, USA.
SO MELAMED, M. R., T. LINDMO AND M. L. MENDELSOHN (ED.). FLOW CYTOMETRY AND SORTING, SECOND EDITION. XII+824P. WILEY-LISS: NEW YORK, NEW YORK, USA; CHICHESTER, ENGLAND, UK. ILLUS. 0 (0). 1990. 531-552. ISBN: 0-471-56235-1
LA English

L1 ANSWER 8 OF 39

AN 90:230452 BIOSIS
DN BR38:108590
TI A FLOW CYTOMETRIC- ***SORTING*** METHOD FOR SEXING MAMMALIAN ***SPERM*** VALIDATED BY DNA ANALYSIS AND LIVE BIRTHS.
AU JOHNSON L A
CS U.S. DEP. AGRIC., ARS, REPRODUCTION LAB., BELTSVILLE, MD. 20705.
SO XIVTH INTERNATIONAL MEETING OF THE SOCIETY FOR ANALYTICAL CYTOLOGY, ASHEVILLE, NORTH CAROLINA, USA, MARCH 18-23, 1990. CYTOMETRY 0 (SUPPL. 4). 1990. 42. CODEN: CYTODQ ISSN: 0196-4763
DT Conference
LA English

L1 ANSWER 11 OF 39

AN 90:27405 BIOSIS
DN BA89:14371
TI OFFSPRING FROM INSEMINATIONS WITH MAMMALIAN SPERM STAINED WITH HOECHST 33342 EITHER WITH OR WITHOUT FLOW CYTOMETRY.
AU MORRELL J M; DRESSER D W
CS NATL. INST. MED. RES., RIDGEWAY, MILL HILL, LONDON NW7 1AA, GREAT BRITAIN.
SO MUTAT RES 224 (2). 1989. 177-184. CODEN: MUREAV ISSN: 0027-5107
LA English
AB The technique of vital staining with a fluorochrome and subsequent flow cytometry has been employed to investigate the DNA content of living sperm. There have been several reports of chromosomal damage caused by staining cells with a bis-benzimidazole dye, Hoechst 33342, either with or without subsequent flow cytometry. Therefore the use of this technique might be expected to affect adversely the fertilising capacity of inseminated sperm, or to result in the production of congenital deformities in the young. Insemination experiments to date have resulted in the production of more than 400 offspring in four species [cattle, pig, rabbit and sheep], including 5 successive generations of rabbits, where stained or ***sorted*** ***sperm*** were used. All progeny were observed to be normal by anatomical criteria. There was no evidence to suggest that sperm treated in this manner were unable to fertilise oocytes in vivo or that embryonic development was affected.

L1 ANSWER 12 OF 39

AN 90:17896 BIOSIS
DN BR38:7196
TI ALTERED SEX RATIOS IN OFFSPRING AFTER SURGICAL INSEMINATION OF FLOW-***SORTED*** POPULATIONS OF X AND Y CHROMOSOME-BEARING ***SPERM*** .
AU JOHNSON L A; FLOOK J P; HAWK H W
CS REPRODUCTION LAB., AGRIC. RES. SERV., U.S. DEP. AGRIC., BELTSVILLE, MD. 20705.
SO TWENTY-SECOND ANNUAL MEETING OF THE SOCIETY FOR THE STUDY OF REPRODUCTION, COLUMBIA, MISSOURI, USA, AUGUST 6-9, 1989. BIOL REPROD 40 (SUPPL. 1). 1989. 162. CODEN: BIREBV ISSN: 0006-3363
DT Conference
LA English

L1 ANSWER 13 OF 39

AN 90:272 BIOSIS
DN BA89:272
TI SEX PRESELECTION IN RABBITS LIVE BIRTHS FROM X AND Y ***SPERM*** SEPARATED BY DNA AND CELL ***SORTING*** .
AU JOHNSON L A; FLOOK J P; HAWK H W
CS REPROD. LAB., BELTSVILLE AGRIC. RES. CENT., AGRIC. RES. SERV., U.S. DEP. AGRIC., BELTSVILLE, MD. 20705, USA.
SO BIOL REPROD 41 (2). 1989. 199-203. CODEN: BIREBV ISSN: 0006-3363
LA English
AB Intact, viable X and Y chromosome-bearing sperm populations of the rabbit were separated according to DNA content with a flow cytometer/cell sorter. Reanalysis for DNA of an aliquot from each

sorted population showed purities of 86% for X-bearing ***sperm*** and 81% for Y-bearing ***sperm*** populations. ***Sorted*** ***sperm*** were surgically inseminated into the uterus of rabbits. From does inseminated with ***sorted*** X-bearing ***sperm*** , 94% of the offspring born were females. From does inseminated with ***sorted*** Y-bearing ***sperm*** from the same ejaculates, 81% of the offspring were males. The probability of the phenotypic sex ratios differing from 50:50 were p < 0.0003 for X- ***sorted*** ***sperm*** and p < 0.004 for Y- ***sorted*** ***sperm*** . Thus, the phenotypic sex ratio at birth was accurately predicted from the flow-cytometrically measured proportion of X- and Y-bearing sperm used for insemination.

L1 ANSWER 17 OF 39

AN 89:69035 BIOSIS
DN BA87:33433
TI FLOW ***SORTING*** OF X AND Y CHROMOSOME-BEARING MAMMALIAN ***SPERM*** ACTIVATION AND PRONUCLEAR DEVELOPMENT OF ***SORTED*** BULL BOAR AND RAM ***SPERM*** MICROINJECTED INTO HAMSTER OOCYTES.
AU JOHNSON L A; CLARKE R N
CS USDA-ARS REPRODUCTION LAB., BELTSVILLE AGRIC. RES. CENT., BUILD. 200, BELTSVILLE, MD. 20705.
SO GAMETE RES 21 (4). 1988. 335-344. CODEN: GAMRDC ISSN: 0148-7280
LA English
AB Flow cytometric techniques were used to measure relative DNA content of X and Y chromosome-bearing bull, boar, and ram sperm populations and to separate the two sex-determining populations. Neat semen was prepared for flow cytometric analysis by washing, light sonication, and staining with 9 .mu.M Hoechst 33342. Computer analysis of the bimodal histograms showed mean X-Y DNA differences of 3.9, 3.7, and 4.2% for bull, boar, and ram, respectively. Flow cytometric reanalysis of ***sorted*** bull, boar, and ram ***sperm*** showed purities greater than 90%. Bull, boar, and ram sperm nuclei were microinjected into hamster oocytes. Microinjected ***sperm*** were either unsorted, ***sorted*** , unsorted plus dithiothreitol (DTT) exposure, or ***sorted*** plus DTT exposure. Following microinjection, eggs were incubated 3 hr, fixed, and stained. A total of 579 eggs was observed for sperm activation (decondensation or formation of a male pronucleus). A lower percentage of ***sorted*** than unsorted (3 vs. 23%) boar ***sperm*** was activated (P < .05). However, ***sorted*** and unsorted DTT-exposed boar ***sperm*** or ***sorted*** and unsorted bull or ram ***sperm*** , regardless of DTT treatment, did not differ significantly. ***Sorted*** ***sperm*** nuclei of both rams and bulls exhibited higher activation rates than ***sorted*** boar ***sperm*** (P < .05). Treatment of ***sperm*** with DTT increased the activation rate (P < .05) for ***sorted*** boar ***sperm*** but not for bull or ram ***sperm*** . These data represent the first separation of bull, boar, and ram X and Y chromosome-bearing ***sperm*** populations and the first evidence that ***sperm*** of domestic animals ***sorted*** on the basis of DNA by flow cytometric procedures have the ability to decondense and to form pronuclei upon injection into a hamster egg.

L1 ANSWER 18 OF 39

AN 88:475562 BIOSIS
DN BR35:105452
TI FLOW ***SORTING*** OF INTACT X AND Y CHROMOSOME-BEARING MAMMALIAN ***SPERMATOZOA*** .
AU JOHNSON L A
CS U.S. DEP. AGRIC., ARS, REPRODUCTION LAB., BELTSVILLE, MD.
SO XII INTERNATIONAL MEETING OF THE SOCIETY FOR ANALYTICAL CYTOLOGY, BRECKENRIDGE, COLORADO, USA, SEPTEMBER 4-9, 1988. CYTOMETRY 0 (SUPPL. 2). 1988. 66. CODEN: CYTODQ ISSN: 0196-4763
DT Conference
LA English

L1 ANSWER 19 OF 39

AN 88:27908 BIOSIS
DN BA85:15633
TI INCIDENCE OF CHROMOSOME ABERRATIONS IN MAMMALIAN ***SPERM*** STAINED WITH HOECHST 33342 AND UV-LASER IRRADIATED DURING FLOWING ***SORTING*** .
AU LIBBUS B L; PERREAULT S D; JOHNSON L A; PINKEL D
CS REPROD. LAB., AGRIC. RES. SERV., U.S. DEP. AGRIC., BELTSVILLE, MD 20705, USA.
SO MUTAT RES 182 (5). 1987. 265-274. CODEN: MUREAV ISSN: 0027-5107
LA English
AB The separation of two ***sperm*** populations is possible using the technique of flow ***sorting*** , provided that a significant difference exists in the DNA content of X- and Y-bearing sperm. In order to ascertain whether or not chromosome damage was induced in ***sorted*** ***sperm*** , chromosome prepartions were made from isolated ***sperm*** that had been microinjected into hamster eggs. While egg chromosomes exhibited a low frequency of chromosome aberrations, ranging from 4 to 7%, a large proportion of sperm cells exhibited chromosome damage. Between 29% of unstained and unsorted sperm and 38% of stained and unsorted sperm exhibited some type of chromosomal abnormality and this proportion increased to 50% in ***sorted*** ***sperm*** . In only damaged ***sperm*** nuclei are considered, the two unsorted sperm groups had a mean of 0.6 breaks, 0.8 triradial exchanges, and 0.2 quadriradial exchanges per nucleus. However, ***sorted*** ***sperm*** , which were stained with a fluorochrome and exposed to UV-laser irradiation, exhibited a mean of 2.9 breaks, 2.6 triradial, and 1.9 quadriradial exchanges per nucleus in which damage occurred. These observations indicate that the treatments and manipulations to which ***sperm*** nuclei are subjected during flow ***sorting*** cause chromosomal aberrations, and that exposure of the cells to UV-laser irradiation contributes substantially to the chromosome damage observed.

L1 ANSWER 20 OF 39

AN 88:17214 BIOSIS
DN BR34:5724
TI SEPARATION OF X AND Y CHROMOSOME-BEARING MAMMALIAN ***SPERM*** BY DNA CONTENT USING FLOW CYTOMETRIC ANALYSIS AND ***SORTING*** .
AU JOHNSON L A
CS U.S. DEP. AGRIC., AGRIC. RES. SERVICE, REPRODUCTION LAB., BELTSVILLE,

MD.
SO SYMPOSIUM ON FETAL-MATERNAL RELATIONSHIPS HELD AT THE TWENTIETH ANNUAL MEETING OF THE SOCIETY FOR THE STUDY OF REPRODUCTION, URBANA, ILLINOIS, USA, JULY 20-23, 1987. BIOL REPROD 36 (SUPPL. 1). 1987. 80. CODEN: BIREBV ISSN: 0006-3363
DT Conference
LA English

L1 ANSWER 22 OF 39

AN 87:414485 BIOSIS
DN BR33:84163
TI FLOW ***SORTING*** OF SONICATED X AND Y CHROMOSOME-BEARING MAMMALIAN ***SPERM*** BASED ON RELATIVE DNA CONTENT.
AU JOHNSON L A; FLOOK J P; CLARKE R N; LOOK M V
CS U.S. DEP. AGRIC., ARS, REPRODUCTION LAB., BELTSVILLE, MD., USA.
SO XIITH INTERNATIONAL MEETING OF THE SOCIETY FOR ANALYTICAL CYTOLOGY, CAMBRIDGE, ENGLAND, UK, AUGUST 9-15, 1987. CYTOMETRY 0 (SUPPL. 1). 1987. 88. CODEN: CYTODQ ISSN: 0196-4763
DT Conference
LA English

L1 ANSWER 23 OF 39

AN 87:385255 BIOSIS
DN BA84:71752
TI FLOW CYTOMETRY OF X AND Y CHROMOSOME-BEARING SPERM FOR DNA USING AN IMPROVED PREPARATION METHOD AND STAINING WITH HOECHST 33342.
AU JOHNSON L A; FLOOK J P; LOOK M V
CS USDA-ARS, ANIMAL SCI. INST., REPRODUCTION LAB., BUILDING 200, BARC-EAST, BELTSVILLE, MD 20705.
SO GAMETE RES 17 (3). 1987. 203-212. CODEN: GAMRDC ISSN: 0148-7280
LA English
AB A new and improved method of preparing mammalian ***spermatozoa*** for high resolution flow cytometric DNA analysis and flow ***sorting*** is described. Ejaculated or cryopreserved ***sperm*** were briefly sonicated to remove tails and then stained with Hoechst 33342. This simple procedure was found superior to more severe treatments of dimethylsulfoxide washes, fixation in 80% ethanol, and protease digestion of the sperm membranes and tails by papain. Flow cytometric DNA analyses of sperm samples subjected to varying sonication times indicted that X and Y chromosome-bearing sperm populations could be well resolved with as little as 15-sec sonication. In addition, a comparison of sonicated samples stained with four concentrations of bisbenzimide (Hoechst 33342) or 4',6-diamidino-2-phenylindole (DAPI) indicated that 2.5 or 5.0 .mu.g/ml of Hoechst was sufficient to resolve the X and Y sperm populations. In order to quantitatively describe the flow cytometric data, several indices (sample quality, orientation and splitting) were developed.

L1 ANSWER 24 OF 39

AN 87:308103 BIOSIS
DN BR33:29776
TI FLOW CYTOMETRY ANALYSIS AND ***SORTING*** OF X AND Y

CHROMOSOME-BEARING ***SPERMATOZOA*** .
AU JOHNSON L A
CS U.S. DEP. AGRIC., REPRODUCTION LAB., ANIMAL SCI. INST., BELTSVILLE AGRIC. RES. CENT., AGRIC. RES. SERV., BELTSVILLE, MD. 20705, USA.
SO AUGUSTINE, P. C., H. D. DANFORTH AND M. R. BAKST (ED.). BELTSVILLE SYMPOSIA IN AGRICULTURAL RESEARCH, 10. BIOTECHNOLOGY FOR SOLVING AGRICULTURAL PROBLEMS; BELTSVILLE, MARYLAND, USA, MAY 5-9, 1985. XIII+416P. MARTINUS NIJHOFF PUBLISHERS: DORDRECHT, NETHERLANDS (DIST. FOR USA AND CANADA BY KLUWER ACADEMIC PUBLISHERS: HINGHAM, MASSACHUSETTS, USA; FOR THE UK AND IRELAND BY KLUWER ACADEMIC PUBLISHERS, MTP PRESS LTD.: LANCASTER, ENGLAND, UK). ILLUS. 0 (0). 1986 (RECD. 1987). 121-134. CODEN: BSARDN ISBN: 90-247-3311-1 ISSN: 0160-3612
LA English

L1 ANSWER 26 OF 39

AN 87:167235 BIOSIS
DN BA83:85676
TI FLOW ***SORTING*** OF X AND Y CHROMOSOME-BEARING ***SPERMATOZOA*** INTO TWO POPULATIONS.
AU JOHNSON L A; FLOOK J P; LOOK M V; PINKEL D
CS USDA-ARS, ANIM. SCI. INST., REPRODUCTION LAB., BUILD. 200, BARC-EAST, BELTSVILLE, MD. 20705.
SO GAMETE RES 16 (1). 1987. 1-10. CODEN: GAMRDC ISSN: 0148-7280
LA English
AB The only established difference on which to base the separation of X and Y chromosome-bearing spermatozoa is chromosomal constitution. This difference is quantifiable both from chromosome morphology (karyotype) and from DNA content. Flow cytometric techniques were used to measure relative DNA content of the X and Y populations and to flow- ***sort*** ***spermatozoa*** from Chinchilla laniger. Epididymal ***spermatozoa*** were recovered in PBS, fixed in 80% ethanol, treated with papain and dithioerythritol, and stained for DNA with Hoechst 33342. ***Sperm*** nuclei were analyzed and ***sorted*** on an EPICS V flow cytometer/cell ***sorter*** , modified specifically for ***spermatozoa*** . Two clearly resolved peaks (coefficient of variation < 1.5%) with approximately 7.5% difference in DNA content between X and Y chromosome-bearing ***spermatozoa*** were evident. ***Sperm*** nuclei were ***sorted*** from a portion of the X and Y peaks at a rate of 55 nuclei/sec for each population. Purities of individual X and Y populations averaged 95% as determined by reanalysis of the ***sorted*** populations. Successful ***sorting*** of Chinchilla X and Y chromosome-bearing ***spermatozoa*** into separate populations may aid in the identification of a biochemical marker that could be used to discriminate between the two sperm populations and lead to a practical procedure for sexing spermatozoa.

L1 ANSWER 27 OF 39

AN 87:35401 BIOSIS
DN BR32:15489
TI FLOW ***SORTING*** OF X AND Y CHROMOSOME BEARING ***SPERM*** INTO SEPARATE POPULATIONS OF THE BASIS OF DNA CONTENT.
AU JOHNSON L A

CS US DEPT. OF AGRICULTURE, AGRIGULTURAL RESEARCH SERVICE, REPRODUCTION LABORATORY, BELTSVILLE, MD. USA.
SO SECOND EUROPEAN CONGRESS ON CELL BIOLOGY, BUDAPEST, HUNGARY, JULY 6-11, 1986. ACTA BIOL HUNG 37 (SUPPL.). 1986. 93. CODEN: ABHUE6 ISSN: 0236-5383
DT Conference
LA English

L1 ANSWER 29 OF 39

AN 86:279451 BIOSIS
DN BA82:23314
TI MODIFICATION OF A LASER-BASED FLOW CYTOMETER FOR HIGH-RESOLUTION DNA ANALYSIS OF MAMMALIAN SPERMATOZOA.
AU JOHNSON L A; PINKEL D
CS USDA-ARS, ANIMAL SCI. INST., REPRODUCTION LAB., BUILD. 200, BARC-EAST BELTSVILLE, MD. 20705.
SO CYTOMETRY 7 (3). 1986. 268-273. CODEN: CYTODQ ISSN: 0196-4763
LA English
AB Modification of a Coulter EPICS V orthogonal laser-based flow cytometer/cell ***sorter*** allows resolution of X and Y mammalian ***sperm*** populations based on DNA content. The modification consists of beveling the sample injection tube situated in the flow chamber, adding a second fluorescence detector directly forward along the laser beam axis, and routing the collected fluorescence through an optical fiber bundle to one of the existing photomultiplier tubes. The X and Y chromosome-bearing spermatozoa from the bull, boar, and ram can be resolved using this system.

L1 ANSWER 33 OF 39

AN 85:136742 BIOSIS
DN BR29:26738
TI FLOW ***SORTING*** OF X AND Y CHROMOSOME-BEARING CHINCHILLA ***SPERMATOZOA*** INTO 2 POPULATIONS.
AU JOHNSON L A; FLOOK J; LOOK M; PINKEL D
CS USDA, AGRIC. RES. SERVICE, REPRODUCTION LAB., BELTSVILLE, MD 20705.
SO 3RD INTERNATIONAL CONGRESS OF ANDROLOGY, BOSTON, MASS., USA, APR. 27-MAY 2, 1985. J ANDROL 6 (2 SUPPL.). 1985. 128-P. CODEN: JOAND3 ISSN: 0196-3635
DT Conference
LA English

L1 ANSWER 34 OF 39

AN 83:318713 BIOSIS
DN BA76:76205
TI FLOW MICRO FLUOROMETRIC ANALYSIS OF LIVING SPERMATOZOA STAINED WITH HOECHST 33342.
AU KEELER K D; MACKENZIE N M; DRESSER D W
CS NATIONAL INST. MED. RES., MILL HILL, LONDON NW7 1AA, UK.
SO J REPROD FERTIL 68 (1). 1983. 205-212. CODEN: JRPFA4 ISSN: 0022-4251
LA English
AB Bovine spermatozoa were stained with Hoechst 33,342. The fluorescence distribution of stained spermatozoa was complex. Non-motile spermatozoa displayed a higher fluorescence than did motile

spermatozoa . The fluorescence profile of the motile ***spermatozoa*** was bimodal. ***Sort*** and re-analysis, and orientation experiments suggested that there are 2 distinct populations of motile spermatozoa.

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=> d 1-23

1. 4,803,297, Feb. 7, 1989, Carbamic acid ester useful for preparing a nucleic acid probe; Corey H. Levenson, et al., 560*159; 548*303

2. 4,777,133, Oct. 11, 1988, Device for quantitative endpoint determination in immunofluorescence using microfluorophotometry; Grace L. Picciolo, et al., 435*29; 250*252.1; 424*3, 7.1; 435*34; 436*46, 511, 527, 531, 537, 800, 807, 826

3. 4,767,703, Aug. 30, 1988, Method for assessing the fertility of male mammals; Roy L. Ax, et al., 435*29, 4, 806; 436*63, 906

4. 4,764,373, Aug. 16, 1988, Method of increasing the economic value of breeding stock semen; Ronald J. Ericsson, 424*105; 435*2

5. 4,754,065, Jun. 28, 1988, Precursor to nucleic acid probe; Corey H. Levenson, et al., 562*564

6. 4,751,313, Jun. 14, 1988, Precursor to nucleic acid probe; Corey H. Levenson, et al., 548*303

7. 4,741,998, May 3, 1988, Monoclonal antibody to MHS-5: a new probe for sexual assault analyses; John C. Herr, et al., 435*7, 28, 70.21, 172.2, 240.27, 810, 948; 436*518, 543, 547, 548, 808; 530*387; 935*104, 108, 110

8. 4,705,886, Nov. 10, 1987, Precursor to nucleic acid probe; Corey H. Levenson, et al., 560*159; 548*303; 562*564

9. 4,643,967, Feb. 17, 1987, Antibody method for lowering risk of susceptibility to HLA-associated diseases in future human generations; Bernard J. Bryant, 435*7; 436*543

10. RE 32,350, Feb. 10, 1987, Thermal convection counter streaming sedimentation and forced convection galvanization method for controlling the sex of mammalian offspring; Bhairab C. Bhattacharya, 204*180.1, 299R

11. 4,622,291, Nov. 11, 1986, Method and device for quantitative end point determination in immunofluorescence using microfluorophotometry; Grace L. Picciolo, et al., 435*4; 250*252.1; 424*3, 7.1; 435*29; 436*46, 527, 531, 537, 800, 807, 826

12. 4,617,261, Oct. 14, 1986, Process for labeling nucleic acids and hybridization probes; Edward L. Sheldon, III, et al., 435*6, 7; 436*94, 501; 548*303; 935*78

13. 4,582,789, Apr. 15, 1986, Process for labeling nucleic acids using psoralen derivatives; Edward L. Sheldon, III, et al., 435*6, 7; 436*501; 935*77, 78

14. 4,559,309, Dec. 17, 1985, Flow cytometry-fluorescence measurements for characterizing sperm; Donald P. Evenson, et al., 436*63; 250*461.2; 436*94, 172

15. 4,448,767, May 15, 1984, Preparation of monospecific male-specific

antibody and the use thereof for increasing the percentage of mammalian offspring of either sex; Bernard J. Bryant, 424*85.9, 105; 435*2

16. 4,397,954, Aug. 9, 1983, Flowcell fractionator; Siddhartha Sarkar, 435*287; 209*158; 435*2

17. 4,360,013, Nov. 23, 1982, Polymeric acid contraceptive devices; Thomas H. Barrows, 128*832; 424*DIG.14; 604*55

18. 4,326,026, Apr. 20, 1982, Method for fractionating cells; Siddhartha Sarkar, 435*2; 424*105; 435*240.1, 240.2

19. 4,191,749, Mar. 4, 1980, Method and material for increasing the percentage of mammalian offspring of either sex; Bernard J. Bryant, 424*105, 85.9, 88; 435*2

20. 4,185,085, Jan. 22, 1980, Differential diagnostic sperm examination; Carsten A. Carstensen, 424*3; 8*506; 424*7.1; 435*2

21. 4,155,831, May 22, 1979, Thermal convection counter streaming sedimentation and forced convection galvanization method and apparatus for controlling the sex of mammalian offspring; Bhairab C. Bhattacharya, 204*299R, 180.1

22. 4,092,229, May 30, 1978, Thermal convection counter streaming sedimentation and forced convection galvanization method for controlling the sex of mammalian offspring; Bhairab C. Bhattacharya, 204*180.1, 299R; 209*11, 173; 435*2, 3

23. 4,083,957, Apr. 11, 1978, Process for the alteration of the sex-ratio of mammals; John L. Lang, 424*78, 79, 80, 81, 82, 83, 105

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1. 4,764,373, Aug. 16, 1988, Method of increasing the economic value of breeding stock semen; Ronald J. Ericsson, 424*105; 435*2

2. 4,511,661, Apr. 16, 1985, ATCC HB8116 And its monoclonal anti-H-Y antibody, Hyclonalan; Ellen H. Goldberg, 436*503; 435*4, 7, 29, 70.21, 172.2, 240.27, 948; 436*501, 510, 518, 519, 547, 548, 815, 821, 824; 530*387, 806, 809; 935*104, 108, 110

3. 4,448,767, May 15, 1984, Preparation of monospecific male-specific antibody and the use thereof for increasing the percentage of mammalian offspring of either sex; Bernard J. Bryant, 424*85.9, 105; 435*2

4. 4,339,434, Jul. 13, 1982, Method of increasing the incidence of female offspring; Ronald J. Ericsson, 424*105; 604*55

5. 4,191,749, Mar. 4, 1980, Method and material for increasing the percentage of mammalian offspring of either sex; Bernard J. Bryant, 424*105, 85.9, 88; 435*2

6. 3,965,300, Jun. 22, 1976, 200 MHz carrier frequency system with sextenary groups; Georg Markl, 370*120

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=> s bisbenzimide(5a)fluorochrome
             1 BISBENZIMIDE
           162 FLUOROCHROME
L2           0 BISBENZIMIDE(5A)FLUOROCHROME

=> s l2 steps
L3   (       1)BISBENZIMIDE
L4   (     162)FLUOROCHROME
L5           0 BISBENZIMIDE(5A)FLUOROCHROME

=> s l3
L6           1 BISBENZIMIDE

=> d
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1. 4,464,470, Aug. 7, 1984, Replication of virulent treponema pallidum in tissue culture; A. Howard Fieldsteel, et al., 435*252.1, 240.23, 240.3, 243, 245

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L1            6 S PRESELECT?(5A)SEX?
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L4 11 BISBENZIMIDE(5A)FLUOROCHROME
L5 4 L4 AND SPERM?
L6 0 L4 AND L1

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L7 0 L3
L8 1 L4

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L8 ANSWER 1 OF 1

AN CA112(5):32693a
TI Radiation sensitivity of tumor cells stained in vitro or in vivo with the bisbenzimide fluorochrome Hoechst 33342
AU Young, S. D.; Hill, R. P.
CS Phys. Div., Ontario Cancer Inst.
LO Toronto, ON M4X 1K9, Can.
SO Br. J. Cancer, 60(5), 715-21
SC 8-6 (Radiation Biochemistry)
SX 14
DT J
CO BJCAAI
IS 0007-0920
PY 1989
LA Eng

1986. 235A. CODEN: JCLBA3 ISSN: 0021-9525
DT Conference
LA English

L5 ANSWER 4 OF 4

AN 87:66135 BIOSIS
DN BA83:34461
TI RAPID VISUAL DETECTION OF ***SPERM*** -EGG FUSION USING THE DNA-SPECIFIC FLUOROCHROME HOECHST-33342.
AU HINKLEY R E; WRIGHT B D; LYNN J W
CS DEP. OF ANATOMY AND CELL BIOL., THE UNIV. OF MIAMI SCH. OF MED., MIAMI, FLORIDA 33101.
SO DEV BIOL 118 (1). 1986. 148-154. CODEN: DEBIAO ISSN: 0012-1606
LA English
AB When unfertilized sea urchin eggs are pretreated with the ***bisbenzimide*** DNA-specific ***fluorochrome*** Hoechst 33342 then washed and fertilized, a single ***sperm*** bound to the egg surface becomes intensely fluorescent. The location of the fluorescent ***sperm*** on the egg surface coincides exactly with the epicenter of the cortical reaction and the site at which the insemination cone subsequently appears. These observations, coupled with studies of eggs treated with quercetin to prevent fusion, as well as eggs made polyspermic by halothane exposure, indicate that the ***sperm*** acquires fluorescence as a consequence of fusion with the fluorochrome preloaded egg. Using a modification of this technique, we have found that cytoplasmic continuity between the ***sperm*** and egg is established at 4-8 sec after the onset of the ***sperm*** -induced conductance increase in the egg.

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FILE BIOSIS
L1 2092 (PRESELECT? OR SELECT?)(5A)SEX?
L2 484 STAIN?(5A)SPERM?
L3 1 L1 AND L2
L4 11 BISBENZIMIDE(5A)FLUOROCHROME
L5 4 L4 AND SPERM?

=> l4 and l1

L6 0 L4 AND L1

fertilized with unlabeled ***sperm*** , a single ***spermatozoan*** bound to the egg surface becomes fluorescent. Several lines of evidence, including correlative scanning electron microscopic studies, indicate that the fluorescent ***sperm*** is, in fact, the fertilizing ***sperm*** which acquires fluorescence as a consequence of membrane fusion between the ***sperm*** and egg. Comparative studies show that several fluorochromes structurally related to H33342 can be used to selectively identify the fertilizing ***sperm*** at the egg surface and that H33258 possesses a distinct advantage when used to visualize the male and female pronuclei in eggs fixed prior to fluorochrome exposure. Finally, none of the fluorochromes tested in these studies have any discernible effect on development from the first cell division through the pluteus larva stage. These observations suggest that the fluorochrome-transfer technique for identifying the fertilizing ***sperm*** may be useful in a wide variety of studies of gamete interaction as a simple and rapid cytological indicator for ***sperm*** -egg fusion.

L5 ANSWER 2 OF 4

AN 87:126822 BIOSIS
DN BA83:65883
TI EFFECTS OF THE VOLATILE ANESTHETIC HALOTHANE ON FERTILIZATION AND EARLY DEVELOPMENT IN THE SEA URCHIN LYTECHINUS-VARIEGATUS EVIDENCE THAT ABNORMAL DEVELOPMENT IF DUE TO POLYSPERMY.
AU HINKLEY R E JR; WRIGHT B D
CS DEP. ANAT. AND CELL BIOL., UNIV. MIAMI SCH. MED., MIAMI, FLA. 33101.
SO TERATOLOGY 34 (3). 1986. 291-302. CODEN: TJADAB ISSN: 0040-3709
LA English
AB The volatile anesthetic halothane, when present at fertilization, dose-dependently increases the incidence of abnormally developing sea urchin embryos at the first cell division. Microscopic examinations of eggs stained with aceto-orcein or the DNA ***fluorochrome*** ***bisbenzimide*** and direct observations on isolated ***sperm*** aster complexes show that halothane induces polyspermy (multiple ***sperm*** entry) when present at fertilization. Experimental evidence suggests that anesthetic-induced polyspermy involves impairment of both the fast (electrically mediated) and slow (morphological) blocks to multiple ***sperm*** entry. These observations clearly show that relatively brief exposures to halothane at fertilization cause polyspermy and that this effect is almost certainly responsible for the ensuing abnormal development observed at the first cell division.

L5 ANSWER 3 OF 4

AN 87:100273 BIOSIS
DN BR32:50074
TI SELECTIVE IDENTIFICATION OF ***SPERM*** FUSED WITH THE SURFACE OF ECHINODERM EGGS USING THE ***BISBENZIMIDE*** DNA-SPECIFIC ***FLUOROCHROME*** HOECHST 33342.
AU HINKLEY R E; WRIGHT B D; LYNN J W
CS DEP. ANATOMY CELL BIOLOGY, UNIV. MIAMI SCH. MED., MIAMI, FL.
SO TWENTY-SIXTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CELL BIOLOGY, WASHINGTON, D.C., USA, DEC. 7-11, 1986. J CELL BIOL 103 (5 PART 2).

L3 ANSWER 1 OF 1

AN 77:161944 BIOSIS
DN BA63:56808
TI ***SEX*** PRE ***SELECTION*** .
AU GLASS R H
SO OBSTET GYNECOL 49 (1). 1977 122-126. CODEN: OBGNAS ISSN: 0029-7844
LA Unavailable

=> d ab

L3 ANSWER 1 OF 1

AB There have been attempts to ***select*** the ***sex*** of a child prior to conception in both animals and humans. Centrifugation, microelectrophoresis and density gradient sedimentation were used to separate X and Y sperm in animals. Only the latter technique has produced a change in sex ratio. In the human, timing of coitus or artificial insemination in relation to ovulation has been the popular method for influencing the sex of a child. The claims of success for coital timing are questioned. Sperm bearing the Y chromosome can now be identified by quinacrine ***staining*** . When ***sperm*** are allowed to swim into columns of liquid albumin a high percentage of Y-bearing sperm are found in the most distal portion of the column. Whether the fraction enriched with Y sperm can produce a preponderance of males is unknown.

=> bisbenzimide(5a)fluorochrome

85 BISBENZIMIDE
1062 FLUOROCHROME
L4 11 BISBENZIMIDE(5A)FLUOROCHROME

=> l4 and sperm?

48953 SPERM?
L5 4 L4 AND SPERM?

=> d 1-4 bib ab

L5 ANSWER 1 OF 4

AN 87:485052 BIOSIS
DN BA84:119695
TI SELECTIVE IDENTIFICATION OF ***SPERM*** FUSED WITH THE SURFACE OF ECHINODERM EGGS BY DNA-SPECIFIC BISBENZIMIDE HOECHST FLUOROCHROMES.
AU HINKLEY R E; EDELSTEIN R N; IVONNET P I
CS DEP. ANATOMY CELL BIOL., UNIV. MIAMI SCH. MED., MIAMI, FL 33101, USA.
SO DEV GROWTH DIFFER 29 (3). 1987. 211-220. CODEN: DGDFA5 ISSN: 0012-1592
LA English
AB When unfertilized echinoderm eggs are treated with the DNA-specific ***bisbenzimide*** ***fluorochrome*** Hoechst 33342 and then

the motile spermatozoa to be sorted into distinct polulations. Non-motile spermatozoa when stained with the dye show a higher fluorescence than the motile spermatozoa. @(9pp Dwg.No.0/6)@

Abstract (GB): 8707 GB2145112

A method of sorting living spermatozoa, the method comprising: the vital staining of spermatozoa with a bisbenzimidazole dye; subjecting the stained spermatozoa to a light source which causes fluorescence; and sorting the spermatozoa according to the fluorescence intensities associated therewith.

File Segment: CPI
Derwent Class: B04; C03; R16
Int Pat Class: C12N-001/00; C12Q-001/68; G01N-021/64;
Manual Codes (CPI/A-N): B04-B02D; B06-D05; B11-C07B; B12-K04; C04-B02D; C06-D05; C11-C07B; C12-K04;
Chemical Fragment Codes (M1):
01 M423 M760 M903 N136 V600 V624
Chemical Fragment Codes (M6):
02 M903 R309 R514 R613 R625 R639

?

using the sepd. X- and Y-bearing sperm populations. @(15pp Dwg.No.0/0)@
File Segment: CPI
Derwent Class: B04; C03; D16;
Int Pat Class: C12N-000/01;
Manual Codes (CPI/A-N): B04-B02D; B04-B04A1; B11-C07B2; B11-C07B3; B12-K04A ; C04-B02D; C04-B04A1; C11-C07B2; C11-C07B3; C12-K04A; D05-H09; D05-H12 ;

8/5/3 (Item 2 from file: 351)
4093376 WPI Acc No: 86-096767/15
XRAM Acc No: C86-041151
Nutritive food obtd. by extn. of nucleic protein from spermaries of fish using centrifuge
Patent Assignee: (NIGY) NICHIRO GYOGO KK
Number of Patents: 001
Patent Family:

CC	Number	Kind	Date	Week	
JP	61040766	A	860227	8615	(Basic)

Priority Data (CC,No,Date): JP 84161041 (840731);
Abstract (Basic): JP61040766
Nucleic protein from spermaries of various sorts of fish is extracted by centrifugation after treatment with water and alcohol and the protein is processed to form dry powder. To eliminate unpleasant odour of genuine spermaries of such kind, the spermaries are preliminarily ground and passed through meshes to form smooth paste.

USE - Spermaries of fishes are economically utilised. @(10pp Dwg.No.0/8)@
File Segment: CPI
Derwent Class: D13; D12;
Int Pat Class: A23L-001/32;
Manual Codes (CPI/A-N): D03-H01T;

8/5/4 (Item 3 from file: 351)
3743426 WPI Acc No: 85-070347/12
XRAM Acc No: C85-030423
Sorting of living spermatozoa by staining with fluorochrome dye and determn. of fluorescence profile
Patent Assignee: (MIMB) MILK MARKETING BOARD
Author (inventor): DRESSER D W; KEELER K D
Number of Patents: 003
Patent Family:

CC	Number	Kind	Date	Week	
GB	2145112	A	850320	8512	(Basic)
GB	2145112	B	870218	8707	
CA	1250808	A	890307	8914	

Priority Data (CC,No,Date): GB 8311451 (830427); CA 480328 (850429);
Abstract (Basic): GB2145112
Living spermatozoa are stored by (1) vital staining with a fluorochrome dye; (2) subjecting the stained spermatozoa to a light source that causes fluorescence; and (3) sorting of the spermatozoa in accordance with the fluorescence associated with them.
USE/ADVANTAGE - The spermatozoa are sorted according to sex, i.e. whether thay bear a X or Y chromosome. N.P. The fluorescence profile of

8/5/1 (Item 1 from file: 345)
5275035
Basic Patent (No,Kind,Date): GB 8311451 A0 830602 <No. of Patents: 003>

PATENT FAMILY:
GREAT BRITAIN (GB)
Patent (No,Kind,Date): GB 8311451 A0 830602
SORTING LIVING SPERMATOZOA (English)
Patent Assignee: MILK MARKETING BOARD
Priority (No,Kind,Date): GB 8311451 A 830427
Applic (No,Kind,Date): GB 8311451 A 830427
IPC: * C12N-001/00
Language of Document: English
Patent (No,Kind,Date): GB 2145112 A1 850320
SORTING LIVING SPERMATOZOA (English)
Patent Assignee: MILK MARKETING BOARD
Author (Inventor): DRESSER DAVID WILSON; KEELER KEITH DAVID
Priority (No,Kind,Date): GB 8311451 A 830427
Applic (No,Kind,Date): GB 8311451 A 830427
National Class: * C6FK
IPC: * C12N-001/00
Derwent WPI Acc No: ; C 85-070347
Language of Document: English
Patent (No,Kind,Date): GB 2145112 B2 870218
SORTING LIVING SPERMATOZOA (English)
Patent Assignee: MILK MARKETING BOARD
Author (Inventor): DRESSER DAVID WILSON; KEELER KEITH DAVID
Priority (No,Kind,Date): GB 8311451 A 830427
Applic (No,Kind,Date): GB 8311451 A 830427
National Class: * C6FK
IPC: * C12N-001/00
Language of Document: English

8/5/2 (Item 1 from file: 351)
5456404 WPI Acc No: 89-378083/51
XRAM Acc No: C89-167614
Predicting sex of offspring - by sepn. of intact viable X and Y chromosome-bearing sperm populations based on DNA content and surgical insemination
Patent Assignee: (USDA) US SEC OF AGRICULTURE
Author (inventor): JOHNSON L A
Number of Patents: 001
Patent Family:
CC Number Kind Date Week
US 7349669 A 891010 8951 (Basic)
Priority Data (CC,No,Date): US 349669 (890510);
Abstract (Basic): US7349669
A method of preselecting the sex of offspring is disclosed comprising (a) separation, by flow sorting, of intact viable X and Y chromosome-bearing rabbit and swine sperm populations based on relative DNA content. (b) surgical insemination of the sorted sperm into does and (c) subsequent birth of sexed offspring with a phenotypic sex ratio consistent with predictions based on the relative DNA content of the sorted sperm populations.
USE/ADVANTAGE - The sex of offspring can be accurately predicted

Derwent Class: B04; P31;
Int Pat Class: A61B-010/00; A61K-039/00;
Manual Codes (CPI/A-N): B04-B02D; B04-B04C; B11-C07A; B12-K04;
Chemical Fragment Codes (M1):
01 M423 M750 M903 N102 P831 V600 V624
02 M423 M781 M903 P831 V600 V611 V791
Chemical Fragment Codes (M6):
03 M903 P831 R515 R521 R621 R622 R639

2/5/12 (Item 1 from file: 350)
1473045 WPI Acc No: 78-35141A/20
XRAM Acc No: C78-A35141
Microscopic differential diagnostic examination of sperm - using pre-stained blood smear microscope slides
Patent Assignee: (BOEF) BOEHRINGER MANNHEIM GMBH
Author (inventor): CARSTENSEN C A
Number of Patents: 005
Patent Family:

CC	Number	Kind	Date	Week	
DE	2651060	A	780511	7820	(Basic)
JP	53086295	A	780729	7835	
DE	2651060	B	791004	7941	
US	4185085	A	800122	8005	
JP	87059264	B	871210	8802	

Priority Data (CC,No,Date): DE 2651060 (761109);
Abstract (Basic): The specimen to be investigated is placed on a pre-stained blood-smear microscope slide and, after development of the staining, examined microscopically. The microscope slides pref. contain methylene blue N and cresyl violet acetate in a proportion of 1:1.5 to 1:5 as their dye components. Development time before the microscopic examination is pref. 5-120 mins.

Used in morphological examination of spermatozoa in andrology, to differentiate between normal and pathological sperm in investigations of fertility. The blood-smear pre-stained slides show up the sperms in good contrast on a colourless background.
File Segment: CPI; EPI
Derwent Class: B04; J04; S03; S05; R16;
Int Pat Class: G01N-033/16; G01N-001/30; G01N-021/60;
Manual Codes (CPI/A-N): B04-B02D; B06-E05; B06-F04; B11-C07B; B12-K04; J04-C04;
Chemical Fragment Codes (M1):
01 V622 V624 V795 N100 M740 M750 P831 P832 M423 M417 M424 M902
Chemical Fragment Codes (M2):
02 K0 H1 M283 M210 M211 M231 M270 M311 M320 C116 E800 L730 L750 H142 H143 N100 M430 M511 M520 M530 M540 M740 M750 P831 P832 M782 R000 M412 M417 M424 M902
03 K0 H1 M282 M210 M211 M231 M240 M270 M281 M311 M320 E540 L350 H142 H143 N100 M430 M511 M520 M530 M540 M740 M750 P831 P832 M782 R000 M412 M417 M424 M902
Ring Index Numbers: 04922
?

Chemical Fragment Codes (M2):
03 C108 D011 D024 D029 D210 G011 G100 H4 H402 H442 H6 H603 H609 H643 H8 J0 J011 J1 J131 K0 L7 L730 M1 M113 M280 M320 M412 M431 M511 M520 M531 M540 M782 M903 M910 N102 P831 Q312 Q505
04 C108 D011 D024 D029 D210 G011 G100 H4 H402 H442 H6 H604 H609 H643 H8 J0 J011 J1 J131 K0 L7 L730 M1 M113 M280 M320 M412 M431 M511 M520 M531 M540 M782 M903 M910 N102 P831 Q312 Q505
05 D022 D023 E210 H1 H100 H103 H142 M210 M211 M240 M273 M281 M282 M320 M412 M431 M511 M520 M530 M540 M640 M782 M903 N102 P831 Q312 Q505
06 C108 D011 D024 D029 D210 G018 G100 H4 H402 H442 H6 H602 H604 H609 H643 H8 J0 J011 J1 J131 K0 L7 L730 M1 M113 M280 M320 M412 M431 M511 M520 M531 M540 M782 M903 M910 N102 P831 Q312 Q505
07 A674 A940 B115 B701 B713 B720 B815 B831 C101 C108 C802 C804 C805 C807 M411 M431 M770 M782 M903 N102 P831 Q312
08 A542 A940 B115 B702 B712 B720 B815 B832 C108 C802 C803 C804 C805 C807 M411 M431 M770 M782 M903 N102 P831 Q312
09 C106 G010 G013 G019 G100 H1 H103 H142 K0 L7 L730 M1 M121 M129 M132 M150 M210 M212 M273 M283 M311 M321 M343 M414 M431 M510 M520 M533 M540 M782 M903 M910 N102 P831 Q312 Q505
10 C106 G010 G013 G019 G100 H1 H103 H142 K0 L7 L730 M1 M121 M129 M132 M150 M210 M211 M273 M283 M311 M321 M343 M414 M431 M510 M520 M533 M540 M782 M903 M910 N102 P831 Q312 Q505
11 C106 G013 G019 G100 H1 H103 H143 K0 L7 L730 M1 M121 M129 M132 M150 M210 M211 M273 M283 M311 M321 M343 M414 M431 M510 M520 M533 M540 M782 M903 M910 N102 P831 Q312 Q505
Chemical Fragment Codes (M6):
02 M903 P831 Q312 Q505 R309 R515 R533 R614 R623 R639
Derwent Registry Numbers: 1360-U; 1384-U; 1427-U; 1934-U; 1990-U; 2003-U

2/5/11 (Item 9 from file: 351)
3195905 WPI Acc No: 83-55911K/23
XRAM Acc No: C83-054453
XRPX Acc No: N83-100462
Determining presence of human sperm by extg. test stain with buffer soln. and developing prostate secretion-globulin by immuno-diffusion in agar
Patent Assignee: (ASTR=) ASTRAKHAN MED INST
Author (inventor): AFANASEVA A V; NIKOLAEV A A; SUNDUKOV V A
Number of Patents: 001
Patent Family:

CC Number	Kind	Date	Week	
SU 946549	A	820730	8323	(Basic)

Priority Data (CC,No,Date): SU 2894533 (800311);
Abstract (Basic): Determn. of the presence of sperm by medicolegal expertise increases the accuracy by: extg. the test stain with buffer contg. 0.1-0.5% Triton X-100 for 18-24 hrs. at 18-20 deg. C; developing prostatic beta-globulin (PBG) in the extract by immunodiffusion in agar.

A positive result indicates the presence of sperm. The proposed method is simpler and quicker than previously. The length of storage of the test stains does not alter the result. Several samples may be tested simultaneously.S Typically, a piece of stained fabric is extd. with 0.1% Triton X-100 soln. for 18 hrs. at 18 deg. C. The extract is tested against a control sample and a sample of test sperm.

Bul.28/30.7.82 (2pp)
File Segment: CPI

the motile spermatozoa to be sorted into distinct polulations. Non-motile spermatozoa when stained with the dye show a higher fluorescence than the motile spermatozoa. @(9pp Dwg.No.0/6)@

Abstract (GB): 8707 GB2145112

A method of sorting living spermatozoa, the method comprising: the vital staining of spermatozoa with a bisbenzimidazole dye; subjecting the stained spermatozoa to a light source which causes fluorescence; and sorting the spermatozoa according to the fluorescence intensities associated therewith.

File Segment: CPI

Derwent Class: B04; C03; R16

Int Pat Class: C12N-001/00; C12Q-001/68; G01N-021/64;

Manual Codes (CPI/A-N): B04-B02D; B06-D05; B11-C07B; B12-K04; C04-B02D; C06-D05; C11-C07B; C12-K04;

Chemical Fragment Codes (M1):

01 M423 M760 M903 N136 V600 V624

Chemical Fragment Codes (M6):

02 M903 R309 R514 R613 R625 R639

2/5/10 (Item 8 from file: 351)

3666916 WPI Acc No: 84-312497/50

XRAM Acc No: C84-133329

XRPX Acc No: N84-233097

Differential staining of mammalian spermatozoa using red dye for nuclear material and green or blue for acrosome

Patent Assignee: (OETT/) OETTLE E E

Author (inventor): OETTLE E E

Number of Patents: 002

Patent Family:

CC	Number	Kind	Date	Week	
ZA	8400224	A	840710	8450	(Basic)
ZA	8400224	A	840710	8445	

Priority Data (CC,No,Date): ZA 831246 (830224); ZA 84224 (840111);

Abstract (Basic): ZA8400224

Method of differential staining of the acrosome and the nuclear material in a sperm head of mammalian spermatozoa contained in a fixed sample comprises (1) treatment of the sample with a red dye to stain the nuclear material red; (2) treatment of the sample with a mordant, and (3) treatment of the sample with a green or blue dye to stain the acrosome of the sperm head green or blue.

USE/ADVANTAGE - The procedure is simple and easily carried out, even by unskilled personnel. The differentiation between the acrosome and the nuclear material is good, so that evaluation of male fertility can be easily determined. The spermatozoa may be from a human or a dog, bear, stallion, ram, bull etc.. (Provisional Basic previously advised in Week 8445) @(19pp Dwg.No.0/0)@

File Segment: CPI; EPI

Derwent Class: B04; C03; D16; S03; R16;

Int Pat Class: G01N-000/00;

Manual Codes (CPI/A-N): B04-B02D; B05-B02A3; B06-A03; B06-D14; B10-B01A; B11-C07B; B12-K04; C04-B02D; C05-B02A3; C06-A03; C06-D14; C10-B01A; C11-C07B; C12-K04; D05-H08;

Manual Codes (EPI/S-X): S03-E13D; S03-E14H9;

Chemical Fragment Codes (M1):

01 M423 M750 M903 N102 P831 V600 V624

Process may further comprise staining a second sample of the sperm with acridine orange and applying flow cytographic measuring techniques to count, simultaneously, the sperm fluorescence emissions at green frequencies 515-575 nm and at red frequencies 600-650 nm, which counts are correlated with cell type and normality.

USE/ADVANTAGE - The process provides a method by which RNA and DNA content/chromatin cndensation as well as cell fertility and motility can all be determined in a rapid, simple and precise manner using flow cytometry. A single measurement can be used to determine (a) percentage of each cell type including (1) mature sperm, (ii) immature sperm precursor cells, (iii) somatic cells e.g. leukocytes, (b) normality/abnormality of sperm nuclear chromatin condensn.. @(7pp Dwg.No.0/2)@

File Segment: CPI; EPI
Derwent Class: B04; J04; S03; S05; R16;
Int Pat Class: G01N-021/64; G01N-033/52;
Manual Codes (CPI/A-N): B04-B02D; B04-B04A1; B06-A03; B06-D13; B11-C07B3; B12-K04A3; J04-C02;
Manual Codes (EPI/S-X): S03-E04A9; S03-E04D; S03-E04H; S03-E14H9; S05-C09;

Chemical Fragment Codes (M1):
03 M423 M750 M903 N102 V624
04 M423 M760 M903 N102 V600 V634
Chemical Fragment Codes (M2):
01 C108 D011 D023 D029 D210 G011 G100 H1 H102 H142 J0 J011 J2 J231 K0 L7 L730 M1 M113 M210 M211 M212 M240 M272 M273 M281 M282 M320 M412 M430 M511 M520 M531 M540 M782 M903 M910 N102 P831 Q505 Q613
02 D013 D022 D029 E120 G010 G100 H1 H101 H142 K0 L7 L721 M1 M113 M210 M212 M273 M281 M320 M412 M430 M511 M520 M531 M540 M782 M903 M910 N102 P831 Q505 Q613
Chemical Fragment Codes (M6):
05 M903 P831 Q505 Q613 R309 R514 R532 R533 R537 R613 R623 R639
Derwent Registry Numbers: 1407-U; 1478-U

2/5/9 (Item 7 from file: 351)
3743426 WPI Acc No: 85-070347/12
XRAM Acc No: C85-030423
Sorting of living spermatozoa by staining with fluorochrome dye and determn. of fluorescence profile
Patent Assignee: (MIMB) MILK MARKETING BOARD
Author (inventor): DRESSER D W; KEELER K D
Number of Patents: 003
Patent Family:

CC	Number	Kind	Date	Week	
GB	2145112	A	850320	8512	(Basic)
GB	2145112	B	870218	8707	
CA	1250808	A	890307	8914	

Priority Data (CC,No,Date): GB 8311451 (830427); CA 480328 (850429);
Abstract (Basic): GB2145112

Living spermatozoa are stored by (1) vital staining with a fluorochrome dye; (2) subjecting the stained spermatozoa to a light source that causes fluorescence; and (3) sorting of the spermatozoa in accordance with the fluorescence associated with them.

USE/ADVANTAGE - The spermatozoa are sorted according to sex, i.e. whether thay bear a X or Y chromosome. N.P. The fluorescence profile of

Patent Family:

CC Number	Kind	Date	Week	
SU 1329780	A	870815	8811	(Basic)

Priority Data (CC,No,Date): SU 4035697 (860324);
Abstract (Basic): SU1329780

The method of determining the quality of spermatozoa involves mixing sperm with a nutrient medium contg. fluorochrome etidium bromide, followed by analysis of the stained spermatozoa under a microscope.

Fluorochrome thiazine red is also introduced into the nutrient medium with the following ratio of ingredients (mass %) fluorochrome thiazine red 0.003-0.012 fluorochrome etidium bromide 0.0005-0.002 and balance nutrient medium.

The quality of the spermatozoa is judged by damage to their head and acrosoma.

ADVANTAGE - This method is determining the quality of spermatozoa is more accurate . Bul.30/15.8.87 @(3pp Dwg.No.0/0)@

File Segment: CPI
Derwent Class: B04; C03; P31; P32;
Int Pat Class: A61B-010/00; A61D-007/02;
Manual Codes (CPI/A-N): B04-B02D; B06-D13; B07-F02; B12-K04A; C04-B02D; C06-D13; C07-F02; C12-K04A;
Chemical Fragment Codes (M1):
01 M423 M750 M903 N102 Q435 V624
Chemical Fragment Codes (M2):
02 D013 D022 D029 E120 G010 G100 H1 H101 H142 K0 L7 L721 M1 M113 M210 M212 M273 M281 M320 M412 M430 M511 M520 M531 M540 M640 M782 M903 M904 M910 N102 P831 Q435 Q505
03 D012 D022 E600 G013 G021 G023 G111 G221 H4 H401 H441 H8 K0 K4 K431 K499 K5 K534 M1 M113 M122 M145 M210 M211 M240 M281 M320 M412 M430 M511 M520 M532 M540 M782 M903 M904 N102 P831 Q435 Q505
Chemical Fragment Codes (M6):
04 M903 P831 Q435 Q505 R309 R514 R533 R613 R623 R639
Derwent Registry Numbers: 1407-U

2/5/8 (Item 6 from file: 351)
4010121 WPI Acc No: 86-013512/02
XRAM Acc No: C86-005735
XRPX Acc No: N86-010122

Characterising sperm motility and viability by flow cytometry-fluorescence measurements after staining

Patent Assignee: (SLOK) MEMORIAL SLOAN KETT
Author (inventor): EVENSON D P; DARZYNKIEW Z
Number of Patents: 001
Patent Family:

CC Number	Kind	Date	Week	
US 4559309	A	851217	8602	(Basic)

Priority Data (CC,No,Date): US 413862 (820901);
Applications (CC,No,Date): US 413867 (820901);
Abstract (Basic): US4559309

Characterisation comprises (a) staining a sample of the sperm with Rhodamine 123 and ethidium bromide, (b) applying flow cytographic measuring techniques to count simultaneously, the sperm fluorescence emissions at green frequencies 515-575 nm and at red frequencies 600-650 nm, the green counts being correlated with sperm motility and the red counts being correlated with putative dying or dead cells.

mixing the sample contg. the salt for a time sufficient to give a satisfactory dispersion of the salt through the sample and to obtain a protein ppte; (d) sepg. the ppte. from the sample; and (e) sepg. the nucleic acid remaining in the sample.

USE/ADVANTAGE - Useful for isolation of RNA and/or DNA for e.g. cell lysates, cell diqests, tissue samples, urine samples, blood stains, spermatozoa, bacterial cells, etc. The method is fast and efficient, and avoids the use of hazardous chemicals such as phenol-CHCl3 which have waste disposal problems and potential for damaging the nucleic acid sample. @(23pp Dwg.No.0/0)@

File Segment: CPI
Derwent Class: B04; D16;
Int Pat Class: C07G-017/00; C07H-015/12; C07K-001/14; C07K-003/12; C12P-019/34;
Manual Codes (CPI/A-N): B04-B04A1; D05-H12; D05-H13;

2/5/6 (Item 4 from file: 351)
5297641 WPI Acc No: 89-219294/30
XRAM Acc No: C89-097635
XRPX Acc No: N89-166996

Laboratory diagnosis of haemospermia - by staining dry ejaculated smear with mixt. of methylene blue, fuchsine, phenol and methanol, rinsing with methanol and microscopy

Patent Assignee: (MOME=) MOSC MED STOMATOLOG
Author (inventor): BRENNER L A; SEGAL A S; TSIMBEROVA P M
Number of Patents: 001
Patent Family:

CC Number	Kind	Date	Week	
SU 1437738	A	881115	8930	(Basic)

Priority Data (CC,No,Date): SU 4160322 (861114);
Abstract (Basic): SU1437738

A dry ejaculate smear is fixed with ethanol and stained with a soln. contg. a 20:5:0.8:1 mixt. of methylene blue, basic fuchsin, phenol and methanol. The stained preparation is then rinsed for 30 sec. in running methanol. Subsequent examination under a microscope reveals the erythrocytes stained red or intense pink, and spermatozoids stained blue. This ensures that an accurate count of erythrocytes can be made.

USE/ADVANTAGE - In urology. More efficient diagnosis is obtd. Bul.42/15.11.88 @(2pp Dwg.No. 0/0)@

File Segment: CPI; EPI
Derwent Class: B04; J04; S03; R16;
Int Pat Class: G01N-001/28;
Manual Codes (CPI/A-N): B04-B04A3; B04-B04D1; B04-B04L; B06-F04; B10-A20; B10-E02; B11-C07B1; B12-K04A; J04-B01;
Manual Codes (EPI/S-X): S03-E13D; S03-E14H;

2/5/7 (Item 5 from file: 351)
4785902 WPI Acc No: 88-076459/11
XRAM Acc No: C88-034565
XRPX Acc No: N88-057846

Sperm quality testing - by additionally introducing thiazine red fluorochrome to nutrient medium

Patent Assignee: (UPOU=) UKR POULTRY FARM IN
Author (inventor): TERESHCHEN A V; SAKHATSKII N I; ARTEMENKO A B
Number of Patents: 001

5355523 WPI Acc No: 89-277178/38
XRAM Acc No: C89-122811
XRPX Acc No: N89-211556
Establishing quality of avian sperm - by adding it to medium contg. rhodamine C and malachite green, preparing smear, and microscopic investigation
Patent Assignee: (UPOU=) UKR POULTRY RES
Author (inventor): BELETSKII E M; REUT I K
Number of Patents: 001
Patent Family:

CC	Number	Kind	Date	Week	
SU	1470293	A	890407	8938	(Basic)

Priority Data (CC,No,Date): SU 4297615 (870710);
Abstract (Basic): SU1470293
Use of Rhodamine C (I) and Malachite green (II) as stains in the testing avian sperm for its quality, increases the efficiency of the test. A drop of sperm is mixed with a drop of a mixt. contg. (in wt.%): (I) 0.05-0.2, (II) 0.15-0.35 and balance nutrient medium, left for 10-15 sec., a smear prepd. and dried with warm (40 deg.C) air. A smear thus prepd. can be stored for several weeks, or sealed in polystyrene and stored for several years. The microscopy under x1250 magnification yields the results.
ADVANTAGE - Increased accuracy of test. Bul. 13/7.4.89. @(3pp Dwg.No.0/0)@
File Segment: CPI; EPI
Derwent Class: A89; B04; C03; S03; P32;
Int Pat Class: A61D-007/02;
Manual Codes (CPI/A-N): A12-V; A12-W04; B04-B02D; B06-A03; B10-A22; B11-C07B1; B12-K04A; C04-B02D; C06-A03; C10-A22; C11-C07B1; C12-K04A;
Manual Codes (EPI/S-X): S03-E09E; S03-E14H9;
Plasdoc Key Serials: 0231 0304 2690 2767 2837
Polymer Fragment Codes (AM):
101 014 04- 055 056 52- 611 645 688 720

2/5/5 (Item 3 from file: 351)
5342026 WPI Acc No: 89-263680/36
XRAM Acc No: C89-117084
Nucleic acid isolation from biological sample - by dissociating protein, precipitating protein with (in)organic salt, and isolating nucleic acid
Patent Assignee: (MEMO-) MEMORIAL BLOOD CENT
Author (inventor): MILLER S A; DYKES D D
Number of Patents: 001
Patent Family:

CC	Number	Kind	Date	Week	
WO	8907603	A	890824	8936	(Basic)

Priority Data (CC,No,Date): US 154024 (880209);
Applications (CC,No,Date): WO 89US463 (890209);
EP and/or WO Language: English
EP and/or WO Cited Patents:
US 4427580; 4.Jnl.REF;
Designated States (National): JP (Regional): AT; BE; CH; DE; FR; GB; IT; LU; NL; SE
Abstract (Basic): WO8907603
Method for isolating nucleic acids from a biological sample comprises: (a) dissociating protein present in the sample; (b) adding sufficient of an organic or inorganic salt to ppte. the protein; (c)

PUTATIVE DYING OR DEAD CELLS.

CA Ref: 104126146
Class: 436063000
Cross Ref: 250461200; 436094000; 436172000
IPC: G01N-021/64
Cross Ref: G01N-033/52

2/5/2 (Item 1 from file: 345)
5867407
Basic Patent (No,Kind,Date): ZA 8400224 A 840829 <No. of Patents: 001>

PATENT FAMILY:
SOUTH AFRICA (ZA)
Patent (No,Kind,Date): ZA 8400224 A 840829
METHOD AND KIT FOR STAINING MAMMALIAN SPERMATOZOA (English)
Patent Assignee: ERIC OETTLE EDMUND; OETTLE EDMUND ERIC
Author (Inventor): ERIC OETTLE EDMUND; OETTLE EDMUND ERIC
Priority (No,Kind,Date): ZA 831246 A 830224
Applic (No,Kind,Date): ZA 84224 A 840111
IPC: * G01N
Derwent WPI Acc No: * C 84-312497
Language of Document: English

2/5/3 (Item 1 from file: 351)
5385878 WPI Acc No: 89-307533/42
XRAM Acc No: C89-136539
XRPX Acc No: N89-234067
Diagnosing ureaplasmose - by preparing smear of semen, fixing, staining with olivomycin, and microscopic examination of green luminescence of DNA in spermatozoa; DEOXYRIBONUCLEIC ACID
Patent Assignee: (KHDE=) KHARK DERMATOLOGY
Author (inventor): MAVROV I I; KARPENKO A E; MIKHAILOVA G R
Number of Patents: 001
Patent Family:

CC Number	Kind	Date	Week	
SU 1448235	A	881230	8942	(Basic)

Priority Data (CC,No,Date): SU 3923521 (850530);
Abstract (Basic): SU1448235
Ureaplasmose (microplasmic damage to sperm) is diagnosed more accurately as follows: A smear or semen is dried in air, fixed in 70% ethanol for 1 hour at 4 deg., stained with soln. of olivomycin, and placed in a darkened chamber for 50-60 min. Brief wash with water is followed by placing a drop of buffered glycerin on the smear, covering it with a cover glass, sealing with paraffin and inspecting for bright green luminescence of DNA in the spermatozoa.
ADVANTAGE - Increased accuracy of diagnosis. Bul.48/30.12.88
@(2pp Dwg.No.0/0)@
File Segment: CPI; EPI
Derwent Class: B04; J04; S03; P31; R16;
Int Pat Class: A61B-010/00; G01N-001/28;
Manual Codes (CPI/A-N): B02-D; B04-B02D; B04-B04A1; B11-C07B4; B12-K04A; J04-B01;
Manual Codes (EPI/S-X): S03-E04E; S03-E09E; S03-E13D; S03-E14H;

2/5/4 (Item 2 from file: 351)

5/5/1 (Item 1 from file: 345)
6467772
Basic Patent (No,Kind,Date): SU 1191454 A1 851115 <No. of Patents: 001>

PATENT FAMILY:
UNION OF THE SOVIET SOCIALIST REPUBLICS (SU)
Patent (No,Kind,Date): SU 1191454 A1 851115
METHOD OF PRODUCING TRANS- AND CIS-ISOMERS OF NAPHTHOYLEN-BISBENZIMIDE OF ASH DYES IN PIGMENT FORM (English)
Author (Inventor): SHIGALEVSKIJ VADIM A (SU); SOLOMATIN GEORGIJ G (SU); BORODINA ANTONINA V (SU); MOROZ VALERIJ A (SU); SHELYAPIN OLEG P (SU)
Priority (No,Kind,Date): SU 3725203 A 840405
Applic (No,Kind,Date): SU 3725203 A 840405
IPC: * C09B-067/48
CA Abstract No: ; 107(16)135887T
Derwent WPI Acc No: ; C 86-143318
Language of Document: Russian
?t s2/5/all

2/5/1 (Item 1 from file: 340)
1641238 8521709
C/ FLOW CYTOMETRY-FLUORESCENCE MEASUREMENTS FOR CHARACTERIZING SPERM; STAINING WITH RHODAMINE 123 AND ETHIDIUM BROMIDE TO DETERMINE MOTILITY AND VIABILITY
Inventors: DARZYNKIEWICZ ZBIGNIEW (US); EVENSON DONALD P (US)
Assignee: MEMORIAL SLOAN KETTERING CANCER CENTER Assignee Codes: 13529
Document Type: UTILITY

	Applic Number	Applic Date	Patent Number	Issue Date
Patent:	US 413862	820901	US 4559309	851217

(Cited in 002 later patents)
Priority Applic: US 413862 820901
Abstract:
A PROCESS FOR CHARACTERIZING SPERM MOTILITY AND VIABILITY BY STAINING A SPERM SAMPLE WITH RHODAMINE 123 AND ETHIDIUM BROMIDE, AND SIMULTANEOUSLY MEASURING THE SPERM FLUORESCENCE EMISSIONS AT GREEN FREQUENCIES 515-575 NM AND AT RED FREQUENCIES 600-650 NM, THE GREEN COUNTS BEING CORRELATED WITH SPERM MOTILITY AND THE RED COUNTS BEING CORRELATED WITH PUTATIVE DYING OR DEAD CELLS. ADDITIONALLY, A SAMPLE OF SPERM CAN BE CHARACTERIZED AS TO TYPE AND NORMALITY BY STAINING A SAMPLE OF SPERM WITH ACRIDINE ORANGE AND SIMULTANEOUSLY MEASURING THE SPERM FLUORESCENCE EMISSIONS AT GREEN FREQUENCIES 515-575 NM AND AT RED FREQUENCIES 600-650 NM.

Claim:

D R A W I N G S

1. PROCESS FOR CHARACTERIZING SPERM MOTILITY AND VIABILITY COMPRISING THE STEPS OF STAINING A SAMPLE OF THE SPERM WITH RHODAMINE 123 AND ETHIDIUM BROMIDE, APPLYING FLOW CYTOGRAPHIC MEASURING TECHNIQUES TO COUNT, SIMULTANEOUSLY, THE SPERM FLUORESCENCE EMISSIONS AT GREEN FREQUENCIES 515-575 NM AND AT RED FREQUENCIES 600-650 NM, THE GREEN COUNTS BEING CORRELATED WITH SPERM MOTILITY AND THE RED COUNTS BEING CORRELATED WITH

US PAT NO: 4,999,283 [IMAGE AVAILABLE] L2: 2 of 92
DATE ISSUED: Mar. 12, 1991
TITLE: Method for x and y spermatozoa separation
INVENTOR: Panayiotis M. Zavos, Lexington, KY
Karl A. Dawson, Lexington, KY
ASSIGNEE: University of Kentucky Research Foundation, Lexington, KY (U.S. corp.)
APPL-NO: 07/396,738
DATE FILED: Aug. 18, 1989
ART-UNIT: 185
PRIM-EXMR: Robin Teskin
LEGAL-REP: King & Schickli

US PAT NO: 4,999,283 [IMAGE AVAILABLE] L2: 2 of 92

ABSTRACT:
A method for separating male and female determining spermatozoa includes the initial step of exposing freshly ejaculated spermatozoa in a substantially protein free diluent to an excess concentration of a monoclonal antibody directed against H-Y antigen that binds substantially exclusively with male determining spermatozoa. The method continues with the suspending of the exposed spermatozoa together with a conjugate of (1) an immunoglobulin G antibody that binds substantially exclusively to the monoclonal antibody and (2) an immunoabsorbant substrate in a substantially protein free diluent. This forms a conjugate/spermatozoa preparation. The method concludes with the recovering of the separated male and female determining spermatozoa.

US PAT NO: 4,764,373 L2: 24 of 92
DATE ISSUED: Aug. 16, 1988
TITLE: Method of increasing the economic value of breeding stock semen
INVENTOR: Ronald J. Ericsson, Ranch in Crook County, WY
ASSIGNEE: Gametrics Limited, Alzada, MT (U.S. corp.)
APPL-NO: 06/802,889
DATE FILED: Nov. 29, 1985
ART-UNIT: 128
PRIM-EXMR: Sam Rosen
LEGAL-REP: Millen & White

US PAT NO: 4,764,373 L2: 24 of 92

ABSTRACT:
Method of artificially inseminating a plurality of animals with aliquots of sperm obtained from a breeding stock individual of that species, obtained by fractionating the collected semen into first and second motile-sperm containing fractions, the first of which is free from immotile sperm and non-sperm components, both suspended in a liquid vehicle which is physiologically acceptable to the sperm and for artificial insemination; dividing the first and second fractions into a plurality of aliquots, each of which contain enough motile sperm to ensure a pregnancy when used for an artificial insemination; artificially inseminating a plurality of individuals of that species in which a

predominance of male offspring is desired with the aliquots of the first fraction; and artificially inseminating a plurality of individuals of that species in which a predominance of male offspring is not a desired objective with the aliquots of the second fraction.

US PAT NO: RE 32,350 L2: 40 of 92
DATE ISSUED: Feb. 10, 1987
TITLE: Thermal convection counter streaming sedimentation and forced convection galvanization method for controlling the sex of mammalian offspring
INVENTOR: Bhairab C. Bhattacharya, 297 Moore St., Princeton, NJ 08540
ASSIGNEE: Bhairab C. Bhattacharya, Princeton, NJ (U.S. indiv.)
Manju Bhattacharya, Princeton, NJ (U.S. indiv.)
APPL-NO: 06/373,143
DATE FILED: Apr. 29, 1982
ART-UNIT: 112
PRIM-EXMR: Howard S. Williams
LEGAL-REP: Bacon & Thomas

US PAT NO: RE 32,350 L2: 40 of 92

ABSTRACT:
A method and apparatus for controlling the sex of mammalian offspring by separation of the X-chromosome female producing sperm and Y-chromosome male producing sperm according to their different characteristics of density of the respective cells and electric potential on the respective cell surfaces. Separation is accomplished by first producing at thermal convection counter stream within a sedimentation column containing a universal medium with sperm suspended therein and allowing the two sperm populations to settle into different fractions according to different densities. Subsequently, the fractions are further separated and concentrated utilizing convection galvanization. The positive and negative geotaxis applied to the sperm during thermal convection sedimentation in combination with galvanic forces applied during the convection galvanization facilitate a more efficient separation than previously obtained. This is due to the fact that a greater degree of separation of X and Y sperm is achieved by subjecting an unbalanced population of sperm cells, i.e., one predominating in X or Y cells, to convection galvanization. Thermal convection counter streaming sedimentation has been found to be a preferred method for attaining this unbalanced sperm population. The apparatus used to accomplish the above separation includes means for producing a temperature differential between axial and peripheral portions of the medium contained in the sedimentation column, thus creating the necessary thermal convection counter stream, as well as an electrophoreses cell comprising a convection column disposed between the two electrodes of the cell. Alternatively, the sedimentation apparatus and the convection galvanization apparatus may be combined. Additionally, the apparatus may comprise a laser capable of scanning the length of the thermal convection sedimentation column as well as laser detecting means to determine the distribution of sperm produced within the medium therein.

US PAT NO: 4,474,875 L2: 51 of 92

DATE ISSUED: Oct. 2, 1984
TITLE: Method and means for controlling the sex of mammalian offspring and product therefor
INVENTOR: Wallace Shrimpton, 320 Judah St., San Francisco, CA 94122
APPL-NO: 06/179,045
DATE FILED: Aug. 18, 1980
ART-UNIT: 125
PRIM-EXMR: Frederick E. Waddell
LEGAL-REP: James F. Mitchell

US PAT NO: 4,474,875 L2: 51 of 92

ABSTRACT:
A method of controlling the sex of mammalian offspring by separating spermatozoa into fractions having the desired sex characteristics and artificially inseminating the female to produce offspring of the desired sex. The sperm is separated by applying a buoyant force or forces to a mixture of sperm in nutrient media so that separation occurs according to density of the sperm. The nutrient media is controlled as to density characteristics and can have a uniform density gradient from top to bottom so that buoyant forces within such media are selectively applied to sperm of different density to effect separation of the sperm and to hold sperm fractions of different density in suspended relation within the nutrient media. Substantially pure sperm fractions (having the desired male or female sex characteristics) are isolated at the top or at the bottom of a separation column for use in artificially inseminating the female. Under certain circumstances, separation of the sperm into fractions is enhanced by the application of gas pressure (positive or negative) above the mixture of sperm and nutrient media in the column.

US PAT NO: 4,339,434 L2: 61 of 92
DATE ISSUED: Jul. 13, 1982
TITLE: Method of increasing the incidence of female offspring
INVENTOR: Ronald J. Ericsson, Sausalito, CA
ASSIGNEE: Gametrics Limited, Sausalito, CA (U.S. corp.)
APPL-NO: 06/293,079
DATE FILED: Aug. 17, 1981
ART-UNIT: 125
PRIM-EXMR: Sam Rosen
LEGAL-REP: Millen & White

US PAT NO: 4,339,434 L2: 61 of 92

ABSTRACT:
The likelihood of conceiving a female fetus is substantially enhanced by promoting ovulation in a fertile female mammal with an ovulation-inducing agent and artificially inseminating the female mammal during the period of expected ovulation with a sperm fraction of enhanced sperm motility from which immotile sperm and non-sperm seminal components have been separated and which is suspended in serum albumin or like physiologically acceptable vehicle.

US PAT NO: 4,276,139 L2: 66 of 92
DATE ISSUED: Jun. 30, 1981

TITLE: Process for magnetic separation and collection of viable female and male spermatozoa
INVENTOR: Rommon L. Lawson, 7905 Bangor Ave., Lubbock, TX 79424
APPL-NO: 06/083,173
DATE FILED: Oct. 9, 1979
ART-UNIT: 116
PRIM-EXMR: G. L. Kaplan
LEGAL-REP: Harvey B. Jacobson

US PAT NO: 4,276,139 L2: 66 of 92

ABSTRACT:
A process for separating and collecting viable female spermatozoa (X-chromosome) and male spermatozoa (Y-chromosome) comprises subjecting a semen sample to an electromagnetic field to cause the female and male spermatozoa to migrate in opposite directions along the direction of the magnetic field. The semen sample is placed in an elongated tube having closed ends and the tube aligned in the direction of the magnetic field whereby the female spermatozoa are collected at the end of the tube in which the magnetic flux of the magnetic field enters and the male spermatozoa are collected at the opposite end of the tube at which the magnetic flux exits.

US PAT NO: 4,244,949 L2: 70 of 92
DATE ISSUED: Jan. 13, 1981
TITLE: Manufacture of long term contraceptive implant
INVENTOR: Gopi N. Gupta, Blauvelt, NY
ASSIGNEE: The Population Council, Inc., New York, NY (U.S. corp.)
APPL-NO: 05/894,088
DATE FILED: Apr. 6, 1978
ART-UNIT: 121
PRIM-EXMR: Henry R. Jiles
ASST-EXMR: Robert C. Whittenbaugh
LEGAL-REP: Brumbaugh, Graves, Donohue & Raymond

US PAT NO: 4,244,949 L2: 70 of 92

ABSTRACT:
This invention relates to a bioabsorbable fused implant for subcutaneous administration in mammals of a constant and effective amount of a steroid over a prolonged period comprising a solid dispersion of an effective amount of a steriod uniformly dispersed in a matrix of a lipoid carrier the weight of steroid to lipoid carrier being within the approximate range of 99:1 to 80:20, respectively, and to the method of manufacturing the fused implants.

US PAT NO: 4,225,405 L2: 71 of 92
DATE ISSUED: Sep. 30, 1980
TITLE: Process for separation and collection of viable female and male spermatozoa
INVENTOR: Rommom L. Lawson, P.O. Box 2111, Lubbock, TX 79408
APPL-NO: 05/933,988
DATE FILED: Aug. 16, 1978
ART-UNIT: 114

PRIM-EXMR: Arthur C. Prescott
LEGAL-REP: Clarence A. O'Brien, Harvey B. Jacobson

US PAT NO: 4,225,405 L2: 71 of 92

ABSTRACT:
A process is disclosed for separating and collecting viable female spermatozoa (XX chromosome) and male spermatozoa (XY chromosome). The process utilizes apparatus comprising two sterilized columns of glass, plastic or other suitable material, a ball valve, a vacuum pump, a mercury manometer, and connecting tubes of glass, plastic or other suitable materials. The system is assembled with rubber percussion gaskets or other suitable connecting materials, to prevent the introduction of extraneous air into the closed system, and a burp bottle to preclude unwanted introduction of fluids into parts of the system under vacuum. The column containing the semen sample may be of variable volume, to accommodate semen samples of varying volume and concentration. The process may be modified by introducing into the system a direct and continuous electrical current flow, or by creating within the separating columns an electrophoretic field. The process may be further modified into a continuous-flow system by adding another mercury manometer, a Cartesian Diver regulator, a second burp bottle, and an over-flow collecting bottle connected to the two main columns.

US PAT NO: 4,191,749 L2: 73 of 92
DATE ISSUED: Mar. 4, 1980
TITLE: Method and material for increasing the percentage of mammalian offspring of either sex
INVENTOR: Bernard J. Bryant, 509 Scripps Dr., Davis, CA 95616
APPL-NO: 05/841,207
DATE FILED: Oct. 11, 1977
ART-UNIT: 125
PRIM-EXMR: Sam Rosen
LEGAL-REP: Mark C. Jacobs

US PAT NO: 4,191,749 L2: 73 of 92

ABSTRACT:
Method, apparatus and material are disclosed for increasing the percentage of mammalian offspring of either sex. The method utilizes a male-specific antibody coupled to a solid-phase immunoabsorbent material to effect a separation of sex-determining spermatazoa derived from a semen suspension. The male-specific antibody selectively binds male-determining spermatazoa. The female-determining spermatazoa are not bound and are recovered directly from the male-specific antibody-coated immunoabsorbent material. The male-determining spermatazoa are recovered from the male-specific antibody-coated immunoabsorbent material after altering the condition thereof to inhibit binding. The apparatus includes a vertical surface comprising beads of immunoabsorbent material over which are distributed male-specific antibodies. The materials are two seminal fluids, one having a substantial preponderance of male-determining spermatazoa, and the other having a substantial preponderance of female-determining spermatazoa.

US PAT NO: 4,155,831 L2: 76 of 92
DATE ISSUED: May 22, 1979
TITLE: Thermal convection counter streaming sedimentation and forced convection galvanization method and apparatus for controlling the sex of mammalian offspring
INVENTOR: Bhairab C. Bhattacharya, 5016 S. 87th St., Omaha, NE 68127
APPL-NO: 05/880,581
DATE FILED: Feb. 23, 1978
ART-UNIT: 114
PRIM-EXMR: Arthur C. Prescott
LEGAL-REP: Bacon & Thomas

US PAT NO: 4,155,831 L2: 76 of 92

ABSTRACT:
A method and apparatus for controlling the sex of mammalian offspring by separation of the X-chromosome female producing sperm and Y-chromosome male producing sperm according to their different characteristics of density of the respective cells and electric potential on the respective cell surfaces. Separation is accomplished by first producing a thermal convection counter stream within a sedimentation column containing a universal medium with sperm suspended therein and allowing the two sperm populations to settle into different fractions according to different densities. Subsequently, the fractions are further separated and concentrated utilizing convection galvanization. The positive and negative geotaxis applied to the sperm during thermal convection sedimentation in combination with galvanic forces applied during the convection galvanization facilitate a more efficient separation than previously obtained. This is due to the fact that a greater degree of separation of X and Y sperm is achieved by subjecting an unbalanced population of sperm cells, i.e., one predominating in X or Y cells, to convection galvanization. Thermal convection counter streaming sedimentation has been found to be a preferred method for attaining this unbalanced sperm population. The apparatus used to accomplish the above separation includes means for producing a temperature differential between axial and peripheral portions of the medium contained in the sedimentation column, thus creating the necessary thermal convection counter stream, as well as an electrophoreses cell comprising a convection column disposed between the two electrodes of the cell. Alternatively, the sedimentation apparatus and the convection galvanization apparatus may be combined. Additionally, the apparatus may comprise a laser capable of scanning the length of the thermal convection sedimentation column as well as laser detecting means to determine the distribution of sperm produced within the medium therein.

US PAT NO: 4,067,965 L2: 79 of 92
DATE ISSUED: Jan. 10, 1978
TITLE: Thermal convection counter streaming sedimentation method for controlling the sex of mammalian offspring
INVENTOR: Bhairab C. Bhattacharya, 5016 S. 87th St., Omaha, NB 68127
APPL-NO: 05/641,501
DATE FILED: Dec. 17, 1975
ART-UNIT: 177
PRIM-EXMR: Tim R. Miles
ASST-EXMR: Ralph J. Hill

LEGAL-REP: Bacon & Thomas

US PAT NO: 4,067,965 L2: 79 of 92

ABSTRACT:
A method and apparatus for controlling the sex of mammalian offspring through separation of X-chromosome female producing sperm and Y-chromosome male producing sperm. The separation is accomplished by producing a thermal convection counter stream within a sedimentation column containing a universal medium with sperm suspended therein, and subsequently allowing the two sperm populations to sediment into different fractions according to different densities. The positive and negative geotaxis thus applied to the sperm facilitate a more efficient separation than has been previously obtained. The apparatus used to accomplish this separation includes means for producing a temperature differential between axial and peripheral portions of the medium contained in the sedimentation column thus creating the necessary thermal convection counter stream, and may also comprise a laser capable of scanning the length of the column and laser detecting means to determine the distribution of sperm produced within the medium.

US PAT NO: 3,894,529 L2: 85 of 92
DATE ISSUED: Jul. 15, 1975
TITLE: Method and means for controlling the sex of mammalian offspring and product therefor
INVENTOR: Wallace Shrimpton, San Francisco, CA
ASSIGNEE: Bio-Controls, Inc., San Francisco, CA (U.S. corp.)
APPL-NO: 04/814,906
DATE FILED: Apr. 10, 1969
ART-UNIT: 335
PRIM-EXMR: Dalton L. Truluck

US PAT NO: 3,894,529 L2: 85 of 92

ABSTRACT:
A method of controlling the sex of mammalian offspring by separating spermatozoa into fractions having the desired sex characteristics and artificially inseminating the female to produce offspring of the desired sex. The sperm is separated by applying a buoyant force or forces to a mixture of sperm in nutrient media so that separation occurs according to density of the sperm. The nutrient media is controlled as to density characteristics and can have a uniform density gradient from top to bottom so that buoyant forces within such media are selectively applied to sperm of differing density to effect separation of the sperm and to hold sperm fractions of different density in suspended relation within the nutrient media. Substantially pure sperm fractions (having the desired male or female sex characteristics) are isolated at the top or at the bottom of a separation column for use in artificially inseminating the female. Under certain circumstances, separation of the sperm into fractions is enhanced by the application of gas pressure (positive or negative) above the mixture of sperm and nutrient media in the column.

US PAT NO: 3,866,598 L2: 86 of 92
DATE ISSUED: Feb. 18, 1975

ABSTRACT:
An immunological method for controlling the sex of mammalian offspring, making use of spermatozoa which has been previously separated into fractions having the desired sex characteristics an antigens. A substantially pure sperm fraction containing the sex chromosomes of a single type (i.e., X chromosomes or Y chromosomes) is introduced into the body of a mammal in sufficient quantity to produce antibodies in the blood stream. A blood sample is then taken from the mammal, the blood coagulated and the blood serum containing the antibodies isolated. Fresh mammalian sperm is inoculated with the blood serum to inactivate and destroy sperm reactive with the antibodies in the blood serum and the treated sperm used to artificially inseminate the female, thereby inducing conception and offspring of desired sex as determined by the remaining unreacted sperm.
In one application of the invention, antibodies reactive with either the X or Y chromosomes may be added to a dose of semen to cause death to sperm containing that type of chromosome before insemination. Alternatively, the antibodies may be introduced into the female either prior or subsequent to copulation (e.g., in a vaginal jelly or as a vaccine) to provide the possibility of **sex** **selection** at conception or possible embryonic death to a fetus of undesired sex.
=>

TITLE: Processes for reproduction of cellular bodies
INVENTOR: Lynn Lawrence Augspurger, 642 Fairfax, Birmingham, MI 48009
APPL-NO: 05/444,022
DATE FILED: Feb. 20, 1974
ART-UNIT: 335
PRIM-EXMR: Aldrich F. Medbery

US PAT NO: 3,866,598 L2: 86 of 92

ABSTRACT:
Disclosed are processes for reproduction of cellular bodies of herbivorous and omnivorous mammals. The techniques include obtaining ova, preparation of recipients and transplant techniques. Methods of **sex** **selection** of spermatozoa are shown as well as several techniques for clonal production of like embryos.

US PAT NO: 3,854,470 L2: 87 of 92
DATE ISSUED: Dec. 17, 1974
TITLE: REPRODUCTION PROCESSES FOR CELLULAR BODIES
INVENTOR: Lynn Lawrence Augspurger, 642 Fairfax, Birmingham, MI 48009
APPL-NO: 05/418,604
DATE FILED: Nov. 23, 1973
ART-UNIT: 335
PRIM-EXMR: Aldrich F. Medbery

US PAT NO: 3,854,470 L2: 87 of 92

ABSTRACT:
Disclosed a process for making greater use of female gametes in herbivorous mannals. The techniques include methods of obtaining ova from donor, preparation of recipients, detection of oestrus, and transplant techniques. Also disclosed are methods of making greater desired use of ova of these mammals. Among those are techniques by sex determination, for freezing and thawing eggs, for tissue culture of eggs and for clonal production of like embroyos utilized for transplantation to achieve a greater number of clonal differentiated cellular bodies having like genetic characteristics.

US PAT NO: 3,692,897 L2: 90 of 92
DATE ISSUED: Sep. 19, 1972
TITLE: IMMUNOLOGICAL METHOD AND COMPOSITION FOR CONTROLLING THE SEX OF MAMMALIAN OFFSPRING
INVENTOR: Bhairab Chandra Bhattacharya, Omaha, NB
Gustaaf J. VAN DEN Bovenkamp, Mill Valley, CA
ASSIGNEE: Bio-Controls, Inc., By said van den Bovenkamp (U.S. corp.)
APPL-NO: 04/873,795
DATE FILED: Nov. 4, 1969
ART-UNIT: 124
PRIM-EXMR: Richard L. Huff
LEGAL-REP: Flehr, Hohbach, Test, Albritton & Herbert

US PAT NO: 3,692,897 L2: 90 of 92